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THE LIFE HISTORY OF A NEW SCHISTOSOME, *SCHISTOSOMATIUM PATHLOCOPTICUM* TANABE,* FOUND IN EXPERIMENTALLY INFECTED MICE

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During the past two years fresh-water snails have been collected in and near Boston, Mass. Among the collected molluscs a species of furcocercous cercaria was found in *Lymnaea palustris*. The morphology and bionomics of this species were studied experimentally. Attempts to infect mice with this cercaria were successful, and consequently the complete life-cycle has been studied. The parasite has proved to be a new species belonging to a new genus, for which the name *Schistosomatium pathlocopticum* Tanabe is proposed.

THE CERCARIA OF SCHISTOSOMATIUM PATHLOCOPTICUM

The cercaria of *Schistosomatium pathlocopticum* (Figs. 1 and 2) was found not only in the digestive gland of four out of one hundred and eighty-six specimens of *Lymnaea palustris* Muell., but also in water containing the infected snails, into which many cercariae escaped. The morphology and bionomics of the cercaria were studied in the free-living stages, in dissected snails, in material fixed with five per cent. formalin, and in sections of infected snails. Intravitam staining was also employed. The finer structures of the cercaria were seen most clearly in living specimens.

The cercaria of *Schistosomatium pathlocopticum* is fork-tailed with a pair of pigmented eyespots. The furcae of the tail are less than half the length of the stem. Measurements of well developed cercariae were made in ten specimens fixed in 5 per cent. formalin. The body averages 0.18 mm. in length by 0.08 mm. in width. The tail-stem measures 0.23 mm. in length by 0.045 mm. in width at the base. The divided lobes are only about 0.1 mm. in length. The whole surface of the

* The specific name *pathlocopticum* was first quoted from Tanabe mss. by Strong (1923:516) in the combination *Schistosoma pathlocopticum;* however, further study has clearly shown that the form can not be included in that genus and a new one must be used for it.

body and of the tail is covered with minute spines. They grow out of a delicate cuticula. Inside this thin membrane there are two layers of muscles; an outer thinner circular and an inner thicker longitudinal layer. A pair of pigmented eyespots is located in front of the middle of the body. They contain minute particles of dark brown pigment. The eyespots measure 8μ in diameter. They are shaped liked a concavo-convex lens. The concave surface is turned toward the side of the body (Fig. 1). Lying just ventrad to the pigmented eyespots is the central nervous system by which both eyes are connected with each other. Several small nuclei surround each eye in a circular arrangement. In the dorsal view of a fresh specimen the eyes seem to be situated in the center of a somewhat polygonal clear area.

The cercaria possesses two suckers: oral and ventral. The oral sucker is large (0.05 mm. in length by 0.047 mm. in transverse section) and well developed. The ventral sucker is much smaller (0.024 mm. in transverse section) than the oral. The oral sucker occupies the anterior third of the body. It is surrounded by muscle fibers which are especially thickened in the posterior portion. The acetabulum is located at a point about one fourth of the distance from the posterior to the anterior end of the body. As seen from the ventral side it is circular. From the lateral aspect it can frequently be seen to protrude like a proboscis (Fig. 2).

Three pairs of cephalic or mucin glands nearly fill the postacetabular region of the cercaria. Two groups of ducts run forward and along the sides of the oral sucker. Each group consists of three ducts which open close together. At the anterior end of the oral sucker there are seen some especially stout spines which point forward. There are three or four of these spines on each side of the middle line. They are hollow and cover the orifices of the two lateral groups of ducts. The shape and location of the cephalic glands are very changeable in the living animal varying with the movements of the body. The ducts are muscular and vary in calibre in different parts of their course. The cephalic glands are acidophil. Fine granules are scattered in the parenchyma of the glands and of the ducts. The glands produce a slimy secretion which is extruded through the ducts and the hollow piercing spines at the anterior end of the body. Frequently a bubble-like mass of this secretion, which is highly refractive, appears at the point of one of the spines.

The digestive system of the cercaria is very rudimentary. The mouth is located on the ventral surface a short distance back of the anterior tip of the body. There is no pharynx. The esophagus is a simple tube. It passes through the oral sucker dorsoposteriorly and extends to the middle of the body, where it divides into two small pockets which are the rudiments of the intestinal ceca of the adult

worm. A group of germ cells is found in the postacetabular region on the median line. The nuclei of the cells are deeply stained by hematoxylin. This group is the basis of the future reproductive organs.

The excretory system of this cercaria consists of six pairs of flame cells, with excretory tubes and capillaries, and a small, oval bladder. The bladder is located at the posterior end of the body proper. Five of the six pairs of the flame cells are found in the body proper. The most anterior flame cell is near the posterior margin of the oral sucker; the second is at the level of the eyespot, the third at the level of the anterior margin of the acetabulum, and the fourth and the fifth are located in the postacetabular region of the body. The sixth is located in the anterior part of the tail. The bladder extends posteriorly through the length of the stem of the tail as a large median tube and divides at the posterior end of the stem into two small tubes which open at the tip of the lobes. At the tip of each lobe the end of its canal is closed by a little bladder apparently astride the tip. In optical section this bladder looks like a pair of claws covering the excretory pore (Fig. 1). The main stem of the tail is round in cross-section, whereas the lobes are somewhat flattened. The tail contains large nuclei in two lateral rows. The stem is set off from the lobes posteriorly and from the body anteriorly by circular constrictions. That one between the body and the tail is especially deep and prominent. The sporocyst of this cercaria is an irregular tube-like body containing numerous cercariae in various stages of development. The wall of the sporocyst is a thin fibrous layer on the inside of which there are scattered nuclei.

According to the system suggested by Cort (1917), the cercaria of *Schistosomatium pathlocopticum* belongs to a group of fork-tailed cercariae, which is characterized by the absence of pharynx, by the presence of eyespots, and by the fact that the tail-stem is more than twice the length of the furci and separated from them by a definite constriction. Such cercariae have been found in various parts of the world. There are several fork-tailed cercariae with eyespots, bearing some gross resemblance to the cercaria of *Schistosomatium pathlocopticum*. They are: *C. ocellata* in Europe and Egypt, *Dicranocercaria ocellifera* in South America, *C. indicae* XLVII and *C. bombayensis* No. 19 in India, *C. echinocauda, C. gigas, C. elephantis,* and *C. douthitti* in North America. Among those cercariae *C. douthitti* Cort (1915, 1918) resembles the most closely in many structural characters the cercaria of *Schistosomatium pathlocopticum*. But they do not agree in the number of the cephalic glands. *C. douthitti* has ten cephalic glands, whereas the cercaria of *Schistosomatium pathlocopticum* possesses only six cephalic glands, three on each side. The latter has the

distinct anterior spines already described which are not reported for *C. douthitti.* From all the evidence obtained it appears that the new cercaria is distinct from any previously described.

BIONOMICS OF CERCARIA OF SCHISTOSOMATIUM PATHLOCOPTICUM

When an infected *Lymnaea palustris* was dissected there was revealed a number of cercariae in various stages of development. In addition to these artificially freed cercariae, there were others which had escaped spontaneously from the infected snails and which were swimming free in the water. They provided excellent material for study of the bionomics of the cercaria. The larva was first found in a jar containing Lymnaeae which had been collected about the middle of October, 1921, from the Back Bay Fens in Boston; the cercariae were first seen on November 25 when there were many of them in the water. When viewed with a hand lens, small, whitish, grayish bodies appeared swimming erratically near the surface of the water, which on being examined microscopically under a greater magnifying power proved to be fork-tailed cercariae with eyespots. Some were floating at the surface of the water, the λ-shaped tail hanging down, and the oral sucker rolling in and out. Others swam about actively in an irregular course, but the swimming was not continuous. Periods of rest for a relatively long time followed periods of active swimming for a short time. The movements of this cercaria consisted of vibration of both body and tail. The cercaria could proceed either forward or backward, but the backward movement was more often observed. When the cercaria found a substance, for instance, algae floating on the surface of water, it would immediately take hold hereof. The cercaria crawled rapidly by the alternate use of both suckers, and frequently by vibrating the tail vigorusly to help the progress of the body. Most of the cercariae were to be found near the surface of the water, as are the cercariae of human schistosomes. The duration of life of this cercaria in ordinary tap water was usually one day. It then sank down to the bottom of the jar, and the body separated from the tail.

A great number of fresh and active cercariae were discharged daily from the infected snails into the surrounding water, especially on cloudy days and at night, or in greater numbers in clear, recently renewed water. Some of this material was used in infection experiments during the period from November 25 to December 21, 1921, on which later date there still survived two infected snails. These were crushed and examined for the cercariae. The snails were still heavily infected. In some instances a piece of the ear of a mouse was cut off and put in the infected water in a Petri-dish under the microscope. The movements of the cercariae became more active as if the larvae were looking for the piece of animal tissue. The cercariae approached

it gradually swimming and looping. When they reached the hair of the ear, they looped quickly along it, passing to the base of the hair and seemed to try to pierce the skin. Stimulated by this characteristic behavior and influenced somewhat by resemblance of the structure of this cercaria to the cercariae of human schistosomes, the animal experiments which follow were performed.

EXPERIMENTAL INFECTION OF MICE WITH THE CERCARIA

On November 26, 1921, the hind legs and the tails of six young mice were immersed for half an hour in water in a jar in which were many active cercariae that had escaped from infected *Lymnaea palustris*. The infection experiment was repeated on December 5. One mouse died on December 24. The autopsy findings were as follows: The liver was yellowish and slightly enlarged. The spleen was also slightly enlarged. The intestines were injected, the mesenteric veins were distinctly engorged, and the mesenteric glands were enlarged. The bladder appeared normal. One immature worm was found in the portal vein. Many ova were found in the tissues of the liver and in the walls of the intestines, but not in the other organs including the bladder.

The second mouse died on December 29, the third on December 30, the fourth on January 3, 1922, and the fifth on January 9. The results of the autopsies were somewhat similar to those of the first one, but the ova found in the livers and in the walls of the intestines were increased in number. There were many adult worms, some in copulation, in the mesenteric veins and the portal vein. Suitable pieces of the tissues were fixed in Zenker's solution, embedded in paraffin, and sectioned at 5μ. The sections were stained either with Giemsa's solution or with Unna's alkaline methylene-blue and eosin (Figs. 38 and 39). The tissues fixed in ten per cent. formalin were cut in celloidin and the sections stained with alum hematoxylin and eosin. The sections stained with slightly alkaline Giemsa solution were excellent.

The mode of infection of the blood flukes of man is dermal. In my previous experiments evidence has already been given in favor of the direct penetration into the skin of mice. In order to obtain definite evidence that the cercaria of *Schistosomatium pathlocopticum* actively penetrate the skin of the final host, the tails of three young mice were immersed in a jar of water containing numerous cercariae by means of a test-tube having a small hole in its bottom through which the tail of the mouse was suspended and came in contact with the infected water. The tails so subjected to immersion for two hours were cut off at the base and subsequently embedded in paraffin. Serial sections were made and stained with Giemsa's solution. The cercariae were found in the act of penetrating the skin

(Figs. 34 and 35). Some were invading the hair-follicles. Others were actively penetrating into the epidermis. As shown in figure 34, the invasion form of the parasite penetrating through the epidermis into the derm, had a collection of dark brown pigment, i. e., an eye-spot, on one side; so that one characteristic structure of the cercaria was still shown even in this stage. Another young parasite was seen in a sebaceous gland (Fig. 35). The cercaria at this stage had lost its tail, although the surface where tail was attached to the posterior end of the body proper was still visible. From this one may infer that the tail had been lost just before penetration of the skin took place.

ATTEMPTS TO INFECT LYMNAEA PALUSTRIS WITH MIRACIDIA

One of the six mice was still alive about three months after infection. A few ova were discharged every day in its feces. When the feces were mixed with tap water, the ova hatched and the miracidia swam about very actively. Attempts were made to produce infection of snails with these miracidia. On January 28, 1922, many snails were collected from Cambridge, where warm water which came out of some occupied houses was stagnating in the sewer. There were many snails, but only *Lymnaea palustris,* no other species being present, congregated in the mud in the winter time so that they were easily collected at this season. Immediately after the collection, thirty-eight Lymnaeae were dissected and examined for furcocercous cercariae. None were found. I selected for the infection experiment forty-seven others, very young and small, among the snails collected, because in my experience such very young snails are not naturally infected. A jar containing water, algae and the snails was kept in a shady part of a room, the temperature of which varied only from 68 to 70 F. during the experiment. The surface of the water was examined for active cercariae for a few days, but no floating or swimming cercaria was observed.

The stools discharged by the mouse were collected in a large dish every day. They were mixed with a small amount of water to make an emulsion and were examined under low magnifying power for the eggs which were present in almost every portion of the stool. The mixture was then put into the aquarium. The experiment was repeated daily for many days. It was very interesting to observe that the snails gathered around the particles of feces and ate them. The particles at first floated on the surface of the water and later sank down to the bottom of the jar; the snails went down for them. In some instances I observed in a Petri-dish under a microscope that the free miracidia attacked the snails very actively and tried to enter the body. On April 7, 1922, about eight weeks after the beginning of the experiment, twenty-six snails survived. All of these were dissected and examined for larval stages of *Schistosomatium pathlocopticum.* In two out of

twenty-six snails were found the same cercariae with sporocysts that had been seen in the snails naturally infected. One showed only the younger stages, rounded and club-shaped forms. The other showed a few fully developed cercariae and many immature larvae. It is, of course, very difficult to be absolutely sure whether or not this infection experiment was successful, for it might be thought that the snails had been already infected naturally. It is most probable, however, that they were experimentally infected, because of the absence of any bifid cercariae in about half the snails collected from the same place at the same time and in the surrounding water observed for a few days after the collection was made. Furthermore, the snails were attacked in a particular manner by the free miracidia, and later they were found infected with the sporocysts and cercariae. To obtain unquestionable results, it would be necessary to secure newly deposited spawn. Such could not be obtained in winter time.

EGG AND MIRACIDIUM OF SCHISTOSOMATIUM PATHLOCOPTICUM

The egg and miracidium of *Schistosomatium pathlocopticum* (Figs. 4-7, 10 and 11) are indeed much like those of *Schistosoma japonicum* in general appearance. The egg of *Schistosomatium pathlocopticum* is oval in shape and has neither spine nor a trace of one. But the surface of the egg-shell does not have a clear-cut outline. The eggs contain miracidia in various stages of development, especially when found in the livers or in the walls of the intestines of infected animals. The eggs discharged by infected hosts are the best material for studying the complete structure. The shell is tough, elastic and permeable. When an egg is placed in water the shell expands, leaving a considerable space between the miracidium and the shell. When the egg is placed in an unfavorable medium, for instance, in a weak solution of formalin, the shell increases in thickness by shrinkage. Within the shell is seen a very thin vitelline membrane. The space between the vitelline membrane and the miracidium is filled with a dense fluid containing oil globules and granules.

The miracidium occupies the center of the egg. Its finer structure is more clearly shown after it has been set free from the shell. When feces containing eggs have been diluted with water and kept at a temperature of 25 C. or slightly higher, the fully developed miracidia in some eggs immediately begin to move, bending the body to right and left, twisting and turning around. At the same time the shell becomes swollen, giving the miracidium a wider space, so that it moves more actively and shows up more clearly. Finally, the shell of the egg splits and the miracidium escapes. The free miracidium swims about, gyrating and pushing aside by means of the anterior papilla any small particles which lay in its way. It undergoes many changes of shape on account of the surrounding conditions. Figure 7 shows a plump form

produced while the miracidium was pushing against a large and resistant mass of débris. Figure 6 shows the usual ovoid form.

With the exception of the anterior extremity or papilla, the entire surface of the miracidia is thickly covered with minute cilia growing out of a thin cuticula. Motion is produced by means of these cilia. From the nonciliated anterior papilla a rudimentary, sac-like intestine can be traced toward the center of the body. On each side of this primitive digestive sac there is a pair of cephalic glands with large nuclei. They communicate with the anterior extremity through ducts similar to those of the cercaria. The central nervous system is an oval mass a little behind the intestinal sac, in about the center of body. Sometimes two oval masses instead of one were seen lying close to each other (Fig. 6). The oval mass is surrounded by a number of deeply stained nuclei as may be seen in sections. This connection is precisely similar to the nervous system of the cercaria, but in the miracidium no pigmented spots are found such as are seen in the cercaria at the center of the mass. One or two large globules are found frequently around the nervous system. A mass of germ cells occupies the posterior half of the body. Four flame cells are found in the anterior and posterior regions, one on each side of the body. Between two flame cells on each side there are small tubes which represent the excretory system of this miracidium. On each side of the latero-posterior part of the body two excretory pores can be made out on careful examination.

The measurements both of mature ova discharged in the feces of the host and of immature ova found in the tissues of the infected mice and in the uterus of the adult female were made on various occasions. Ten mature ova containing fully developed miracidia measured 80 to 126μ in length by 58 to 87μ in breadth. They average 96μ in length by 68μ in breadth. As previously stated, the shell is enlarged when the eggs are placed in water. The miracidia also became enlarged. They measured 116 to 128μ in length by 78 to 84μ in breadth. These measurements were made when the miracidia had ceased their activity after the addition of a weak solution of formalin to the water. The eggs found in the wall of the intestine in infected mice were smaller in size, measuring 49 to 75μ in length by 38 to 60μ in width. In the liver they measured 56 to 98μ in length by 43 to 70μ in width. As seen in the uterus of the adult female, the ova were more or less oval in shape and never had any spines on the shell. They did not yet show differentiation of the miracidium and they were filled with embryonal cells. They measured from 32 to 53μ in length by from 27 to 43μ in width. Therefore, the eggs develop and the miracidia are differentiated during their passage through the tissues after they have been deposited by the female in the branches of the vein.

THE ADULT WORMS OF SCHISTOSOMATIUM PATHLOCOPTICUM

Five of the six young mice, subjected twice to infection with the cercariae died during the period of from 29 to 45 days after the first infection. Another which survived was killed after about five months. In all these six cases numerous adult worms (Figs. 8, 9, 12-33) were obtained from the portal vein and its mesenteric branches. Some were in copulation and others separate. My observations were made from mounts of the entire parasite selected from this material and also from sections of tissues which were fixed in Zenker's solution and stained with Giemsa's solution. The adult worms are dioecious trematodes belonging to the family Schistosomatidae. The male is longer than the female.

THE MALE

The male has a grayish-white, cylindrical body measuring 5.6 to 11.8 mm. in length by 0.4 to 0.9 mm. in width. The body of the male consists of an anterior flattened portion and a posterior larger portion two sides of which are ventrally infolded. By this ventral infolding of the sides a gynecophoric canal is formed in which the female is partially enclosed when the worms are in copula. The anterior flattened portion measured from 2.4 to 4.7 mm. in length by about 0.26 to 0.58 mm. in breadth. The posterior infolded portion measured from 3.2 to 7.1 mm. This part is relatively wide. The largest specimen measured 0.74 mm. in width at the posterior half. In some instances the infolded sides of the posterior part of a worm of medium size were forced apart to show the real leaf-like body shape of trematode. It measured 1.04 mm. in breadth. At the posterior extremity the infolded sides end somewhat abruptly. There is a definite constriction between the anterior and the posterior portion, at which the gynecophoric canal begins. This constricted narrow part measured 0.24 to 0.41 mm. in width.

The integument of the parasite is smooth, whereas in many species of schistosome it is tuberculated. Under higher magnifying power, however, both the outer surface of the body and the inner surface of the gynecophoric canal are found to be closely beset with delicate spines. In cross-section the cuticula is thin and homogeneous throughout. Beneath the cuticula are found three layers of muscles; a circular, a longitudinal, and an oblique muscular layer. In addition to these, there are muscular fibers which run dorsoventrally through areolar connective tissue. The parenchyma of the parasite consists of areolar tissue composed of sustentacular cells, the nuclei of which are rounded and stain deeply. In cross-section (Figs. 14-25) the thickness of the body becomes gradually less toward the edges of the gynecophoric canal. The diminution is due to the decreasing amount of alveolar

connective tissue. The sides overlap one another. The lateral margin on one side is blunt and rounded. That on the other side tapers more extensively and is pointed. The body is not symmetrical.

The parasite possesses two suckers: an oral and a ventral, just like the cercaria. But the ventral sucker of the adult male (0.25 to 0.29 mm. in diameter) is much larger than the oral (0.13 to 0.16 mm. in diameter). The smaller size of the oral sucker is an important characteristic for differentiation. The oral sucker occupies an oblique position. Its dorsal lip is longer than the ventral, the former being the most anterior part of the parasite. The rounded rim is thick and muscular. The oral cavity is infundibular in form and closely beset with spines. The internal opening of this cavity is somewhat pointed and terminate at the esophagus. The esophagus is a simple tube measuring about 0.52 mm. in length. It presents slight constrictions near the acetabulum, just in front of which the esophagus bifurcates into two intestinal tubes. The intestinal branches run backward in a zigzag course, lying somewhat on either side of the midline. In an adult worm having a body length of 6.82 mm., the intestinal branches unite again into a medium tube at a point 0.6 mm. in front of the caudal extremity. This united gut is short and terminates about 0.14 mm. in front of the posterior end of the body. Speaking in general, it is noteworthy that the united gut of this species occupies less than one tenth of the total body length. Among schistosomes *Schistosoma japonicum* has a relatively short cecum occupying one quarter to one fifth, sometimes one sixth, of the total body length. Therefore *Schistosomatium pathlocopticum* has an extremely short cecum.

The excretory system consists of two small longitudinal canals which are seen on either side of the cecum. They unite to form a common tube which opens exteriorly at the extreme posterior edge of the parasite and somewhat dorsally where lies the excretory pore. The testes are situated at the anterior part of the gynecophoric canal, and far back of the ventral sucker. They are located above the intestinal ceca, and just beneath the dorsal surface. The testes, which are darker in color than the rest of the body, appear as a certain number of small rounded or lobulated bodies lying in two parallel rows. There are about fourteen to eighteen, usually sixteen in number. The content of each lobe is coarsely granular. The bodies measure from 0.1 mm. to 0.18 mm. in diameter. By an equal number of vasa efferentia the testes communicate with a large seminal vesicle which lies on the left side of the body. The seminal visicle is roughly semilunar in outline, the concavity being ventromedian and the anterior arm of the arc curved median and backward to receive the vas deferens, the posterior arm being directed median and forward as the ductus ejaculatorius, so crossing the vas deferens at right angles (Figs. 8 and 13). The geni-

tal pore situated at the midline or a little toward the left side of the beginning part of the gynecophoric canal. The situation and number of testes are characteristic of this species. In these respects it differs from other species of schistosome.

THE FEMALE

The female is smaller, more filiform and darker in color than the male. It was found either in the gynecophoric canal of the male or lying alone in the blood vessel of the final host, in which frequently it assumed a curved position. The approximate body length of the female was from 4.5 to 10.2 mm. Often it was difficult to obtain the exact measurement, because of the curved position. In general, the female of this species is shorter than the male. This is a notable difference from the schistosomes of man and of some animals. The body widens from the anterior end to the posterior. The width of the posterior half is from 0.18 to 0.38 mm. The caudal extremity is blunt and terminates abruptly. Both suckers are weak and rudimentary, compared with those of the male. Behind the ventral sucker is the vulva which is sometimes prominent and protuberant. Delicate spines are visible on the anterior part of the body, and especially around the suckers and the vulva.

As above mentioned, the course of the alimentary canal of this species is particularly characteristic. That of the female is substantially the same as that of the male. A simple esophagus bifurcates in front of the acetabulum to form the intestinal ceca which pass backward along both sides of the uterus and the ovary up to the posterior part of the body. About 0.5 mm. in front of the posterior end in a female worm of medium size, they unite to form a common cecum which terminate blindly near the posterior extremity of the parasite. The two branches of the intestine, however, lie nearer to one another from the posterior end of the ovary until they unite. The excretory system is almost the same as in the male.

The ovary is an elongated oval organ lying in the anterior half of the body. The vitelline glands are lobulated and densely packed, and occupy almost all the space between the ovary and the posterior termination of the parasite. The shell gland or Mehlis' gland consists of large, ill-defined cells situated in front of the ovary, where it joins the oviduct with the vitelline duct and with the uterus. The latter is a long tube situated between the shell gland and the vulva. Its length is about 0.5 mm., and it is filled with a great number of yellowish ova. The ova are somewhat rounded in shape. The larger ones are situated near the genital pore; they measure about 40 by 59μ. All are immature and show no embryonal differentiation. They never have spines.

DEVELOPMENTAL STAGES OF ADULT IN FINAL HOST

According to Leiper, the bilharzia worms reared experimentally from cercariae do not attain in laboratory animals the full growth met with in their natural hosts. They are young and small, although sexually mature and actively producing eggs. Therefore, differential characters should be based upon specimens secured under the same circumstances if possible.

Cort (1921) studied the development of the Japanese blood-fluke in mice. His observations are the best available for comparison. The writer on June 4, 1922, in order to compare the series of developmental stages of *Schistosomatium pathlocopticum* with those of *Schistosoma japonicum* described by Cort, exposed twelve mice to infection with cercariae of *Schistosomatium pathlocopticum*. These mice were examined on the twelfth, fourteenth, eighteenth, twenty-fourth and thirtieth day after the exposure to infection. In this experiment, unfortunately, worms of only one sex, male, developed in the livers of the mice.

Another series of six mice were subsequently infected twice, with a lapse of nine days between exposures, namely, on June 5, with cercariae escaping from the same snail used as in the former series, and on June 14, with cercariae discharged by another infected *Lymnaea palustris*. One of the six mice died on July 5, thirty days after the first exposure to infection. Many sexually mature males and nearly mature female worms were found in the liver and in the mesenteric veins. Finding of the female in this series may be interpreted as the result of the second exposure, for only male worms were developed from the first exposure of twelve mice in the first series. These infection experiments indicate that a sexual dimorphism is present in the cercarial stage, or perhaps even earlier in the egg. The accompanying illustrations (Figs. 26-33) show the series of the developmental stages, and Table 1 the measurements.

It may be seen from the figures and the table that the greater size of the male of *Schistosomatium pathlocopticum* is very marked during the early period, but at later stages there are not striking differences in size between that species and *Schistosoma japonicum*. Both species attain sexual maturity about three weeks after infection, and there are not any remarkable differences of total body length or of the width of preacetabular region. However, the ratio of length to width in *Schistosomatium pathlocopticum* is much greater than in *Schistosoma japonicum*, that is, the former is comparatively slender, while the latter is wider. One of the most noticeable features of the male of *Schistosomatium pathlocopticum* is a narrow constriction in the postacetabular region. In the very young, premature stages this constriction is located at the posterior half of the body. But the posterior half of

body grows more rapidly than the anterior. Thus at later stages of development the constriction is situated in front of the middle of body. The ventral infolding of the margins to form the gynecophoric canal starts from this constriction about two weeks after infection. It extends to the posterior extremity. At this stage or a little later several rudiments of testes are visible at region a little back of the constriction. They differentiate to rounded or lobulated bodies. In the vast majority of the sexually mature males, there are seen sixteen testes.

The development of the intestinal ceca up to the union takes a markedly longer time in *Schistosomatium pathlocopticum* than in *Schistosoma japonicum*. In the latter the lateral branches of intestine have united two weeks after infection, or speaking in general, at the sexually premature stage, while in *Schistosomatium pathlocopticum*

TABLE 1.—*Development Stages of Schistosomatium pathlocopticum*
All Measurements in mm.

How many days after infection?..	12	14	18	21	24	30	21
Male or female?.....	M	M	M	M	M	M	F
Length of body.....	1.658	2.633	3.57	3.85	5.785	6.338	3.851
Width of ventral sucker.....	0.384	0.455	0.565	0.486	0.486	0.520	0.195
Width at middle of preacetabular region	0.293	0.423	0.471	0.406	0.423	0.504	0.151
Width at middle of postacetabular region	0.228	0.356	0.356	0.520	0.461	0.57	0.276
Length of preacetabular region...	0.39	0.461	0.683	0.715	1.008	1.998	0.39
Per cent. of total length taken up by preacetabular region.....	23.0	18.0	19.0	19.0	17.3	15.7	10.0
Ratio of length to width.....	40:1	58:1	62:1	95:1	119:1	122:1	20:1
Length of oral sucker.....	0.104	0.117	0.13	0.132	0.151	0.166	0.98
Width of oral sucker.....	0.098	0.111	0.127	0.127	0.145	0.151	0.845
Length of ventral sucker.....	0.195	0.228	0.231	0.244	0.26	0.276	0.13
Width of ventral sucker.....	0.175	0.228	0.215	0.228	0.13	0.151	0 114
Length of bifurcated part of intestine	1.105	1.983	2.715	3.87	4.501	5.131	3.52
Length of united part of intestine	0	0	0	0	0.293	0.406	0.228
Width of intestine.....	0.021	0.021	0.033	0.04	0.057	0.057	0.065
Constriction in postacetabular region {from acetabulum		0.406	0.683	0.861	0.975	1.138	1.332
{from posterior end.....		0.634	0.883	1.641	1.983	3.429	3.82
{width		0.211	1.64	0.325	0.325	0.423	0 356

the two lateral branches are hardly united into a common cecum when sexual maturity is attained. In other words, the lateral branches of the gut are lengthy and the cecum is correspondingly short in *Schistosomatium pathlocopticum*. *Schistosoma japonicum* itself has a short cecum, compared with other blood-flukes. *Schistosomatium pathlocopticum* possesses, therefore, an extremely short cecum. The gynecophoric canal of the male extends from the constriction to the posterior extremity. Corresponding to this relatively short canal the female is shorter than the male.

DIAGNOSTIC DESCRIPTION OF SCHISTOSOMATIUM nov. gen.

Generic Diagnosis: Characters of Schistosomatidae; male (6 mm. in length by 0.4 mm. in width) larger and longer than female (4.5 mm. in length by 0.18 mm. in width); oral sucker small, ventral sucker larger, well developed.

Male: anterior flattened portion occupies anterior two-fifths of body and is constricted from posterior infolded portion, gynecophoric canal; testes situated just behind constriction, arranged in two parallel rows, 14-18, usually 16 in number; intestinal branches unite near posterior end of body.

Female: ovary situated in anterior half of body; intestinal branches unite near posterior extremity of body as in male; uterus contains large numbers of rounded oval, spineless eggs.

Characters of larval stage: furcocercous cercaria having a pair of pigmented eyespots and excretory system consisting of oval bladder, tubes, and six pairs of flame cells, one pair of which is located in base of tail; host, *Lymnaea palustris* Muell. from Boston and Cambridge, Mass., U. S. A.

Natural host of adult, unknown, experimentally grown in mice and white rats.

Type species; *Schistosomatium pathlocopticum.*

The following table shows differences between the new genus, Schistosomatium, and the already established genera. They are Schistosoma Weinland 1858, Bilharziella Looss 1899, Gigantobilharzia Odhner 1910, Ornithobilharzia Odhner 1912, and Austrobilharzia Johnstone 1916.

The Differential Table Between the Six Genera of the Family Schistosomatidae

Genus	Schistosomatium	Schistosoma	Austrobilharzia	Ornithobilharzia	Gigantobilharzia	Bilharziella
Comparative size of male and female	Female shorter than male	Female longer than male, except S. turkestanicum	Female longer than male	Female shorter than male	Female shorter than male	Female shorter than male
Suckers	Present	Present	Two in male, one in female	Present	Absent	Present or absent
Anterior region of male	Long	Short	Short	Short	Short	Short
Testes	Not numerous, 14–18	Less than 10 in most species; 61, 80 in some species	Not numerous, 18–20	Numerous, 90–110	Numerous, near 100 or more	Numerous, 110 or more
Place of ovary and of union of intestinal ceca	Ovary situated in anterior half. Intestinal ceca unite near posterior extremity	Anterior, middle or posterior; ovary situated in front of union of intestinal ceca	Posterior	Anterior	Anterior	About middle
Eggs in uterus	Numerous	Numerous, except in few species	One	One	One ?	One
Character of cercaria	Forked-tailed, with eyespots	Forked-tailed, without eyespots; so far as been known	Unknown	Unknown	Unknown	Unknown

Thanks are due to Doctors R. P. Strong, G. C. Shattuck, E. E. Tyzzer, H. B. Ward, and W. W. Cort through whose courtesies this work has been brought to completion.

SUMMARY

1. A furcocercous cercaria with a pair of pigmented eyespots was found in *Lymnaea palustris* Müll. collected at Boston, Mass. during 1921 and 1922.

2. Series of young mice were exposed with success to infection with this cercaria.

3. Experimental investigations during the last two years show that the free cercariae penetrate actively the skin of the experimental hosts, mice and white rats, and proceed to the liver, where in about one month they attain sexual maturity. Eggs are deposited by the female in the portal vein and its mesenteric branches.

4. Eggs pass to the intestinal canal and are discharged in the feces. They are rounded oval, and spineless. When the stools are mixed with water, miracidia hatch out and swim about in water. They enter *Lymnaea palustris* and develop into sporocysts which produce cercariae in about two months.

5. The adult worms, male and female, have the essential shape of a schistosome. Distinctive structures of the adult worms and the specific larval stage show that this trematode belongs to a new genus and species in the Schistosomatidae for which the name *Schistosomatium pathlocopticum* is proposed.

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Abreviations

a, acetabulum
ax, anterior excretory pore of miracidium
b, bladder
cg, cephalic glands
e, eyespot
et, excretory tube
ex, excretory pore of cercaria or adult
f, flame cell
g, germ cell
gp, genital pore
i, digestive system
m, mouth
o ovary
od, oviduct
og, oil globule
p, papilla
px, posterior excretory pore of miracicium
s, spines
sg, shell gland
t, testes
u, uterus
v, vitellaria
vd, vitelline duct
vm, vitelline membrane
vr, vitelline reservoir

Explanation of Plates

All figures concern *Schistosomatium pathlocopticum*.

Explanation of Plate XIV

Fig. 1.—Cercaria, ventral view. × about 450.

Fig. 2.—Cercaria, lateral view. × about 450.

Fig. 3.—Cercaria in various stages of contraction. × about 90.

OBSERVATIONS ON THE MORPHOLOGY AND LIFE HISTORY OF *HERPETOMONAS MUSCAE-DOMESTICAE* IN NORTH AMERICAN MUSCOID FLIES *

ELERY R. BECKER

Certain flagellate parasites of insects are of particular interest to investigators because of both their general biological importance and the relationships which they bear to mammalian parasites belonging to the genera Leishmania and Trypanosoma. Perhaps the commonest and most extensively investigated of all these insect flagellates is *Herpetomonas muscae-domesticae,* a parasite of the common muscoid flies. No attempt will be made to give an extensive review of the work of previous investigators, since it would make this contribution unnecessarily lengthy. The references at the end of this paper, particularly those of Wenyon (1913) and Brug (1914), should be consulted for publications referred to in this paper and not listed in the bibliography.

The writer has encountered three distinct morphological types of Herpetomonas in the muscoid flies of North America (Text fig. A). (1.) The type that is unmistakably the *Herpetomonas muscae-domesticae* which Prowazek (1904) described, whose distinctive features are strongly staining flagella, each flagellum thickened at the margin of the cell body so as to form marginal granules, and a large deeply staining parabasal body. This flagellate is almost always found in a state of division, making it appear biflagellated. During the summer of 1922 this species was found to be present in varying percentages in the alimentary canal of *Phormia regina, Lucilia sericata, Calliphora erythrocephala, Cochliomyia (Chrysomyia) macellaria, Musca domestica* and *Sarcophaga bullata.* The *Herpetomonas* found in these six species of flies were morphologically indistinguishable, and exhibited no more morphological variations in hosts of different species than in different hosts of the same species. In a later paper will appear a report on cross-infection experiments which seems to indicate that *H. muscae-domesticae* is the common parasite of these six species of flies.

(2.) In *Muscina stabulans, Calliphora erythrocephala,* and *Limosina* sp. is a herpetomonad of somewhat smaller size with a flagellum and parabasal body less strongly developed than in *H. muscae-domesticae.*

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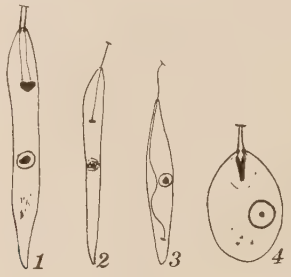
It corresponds very well with the *Crithidia calliphorae* of Swellengrebel (1911), although there is no reason why it should be placed in the genus Crithidia. (3.) In two individuals of a number of *Sarcophaga securifera* examined, a Herpetomonas quite different from any yet described was found. Its cell body was much rounded, and in the stained preparations showed a deeply staining flagellum, prominent marginal granules, a deeply staining parabasal body surrounded by a large clear space, and a nucleus with a very small karyosome and a pronounced achromatinic nuclear membrane (Fig. 1). The first type of Herpetomonas, however, is by far the most common. *Herpetomonas muscae-domesticae* in the host *Phormia regina* was used for the most part in the investigations recorded in this paper. Of two hundred "wild" flies of this species captured during the month of June, 1922, sixty-six per cent. were infected.



Text fig. A.—The three types of Herpetomonas found in North American muscoid flies. 1, *H. muscae-domesticae*; 2 and 3, forms from *Muscina stabulans;* 4, Herpetomonas found in *Sarcophaga securifera.*

The seat of infection is the alimentary canal where the parasites may be distributed from the crop to the rectum. Strickland (1911) made a study of the stages of the parasite in the different regions of the alimentary tract of a species of Lucilia. The fact that infections vary much in their nature should not be overlooked, for in some cases in which the entire intestine swarms with flagellates it is difficult to find any except the long flagellated forms. In another type of infection the crop and anterior end of the midgut may be free from parasites, while the posterior portion of the midgut and the hindgut may contain numerous stumpy flagellates, cysts, and encysting forms. I have rarely found the stumpy and encysting forms in the crop. When herpetomonads are present in the crop, they are generally the long flagellated forms known as "adults" (Figs. 2-15).

A series of fifty "adult" herpetomonads taken ten each from five heavily infected flies gave the average measurements indicated in

Table 1. The preparations used in making the measurements were fixed in Schaudinn's fluid (without acetic acid) and stained by Heidenhain's iron-hematoxylin method. A comparison with dried smears stained by the Romanowsky stains showed that the length of the cell body of dried individuals averaged about 5.0μ greater than in the case of those in the "wet mounts."

From this table it can be seen that there is considerable variation in the measurements of this type of the flagellate in various hosts of the same species; e. g., the average total length in one fly was 55.1μ, while in another fly it was 33.6μ. One monster (not considered in above averages) was encountered with a total length of 79.9μ, the body length alone being 40.1μ (Fig. 15). In general the flagellate ranges in length from 25.0 to 79.9μ, although by far the greater number of individuals measure in the proximity of 45.0μ in length and from 1.5 to 2.5μ in width.

TABLE 1.—*Measurements in microns of fifty adult herpetomonads taken at random ten each from five infected flies*

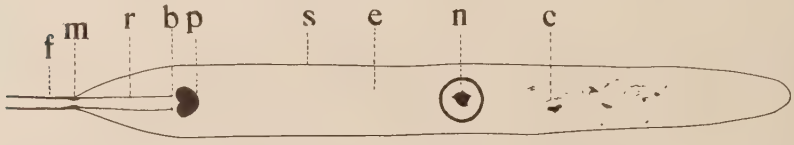
Slide number	Posterior tip to nucleus	Nucleus to marginal granules	Posterior tip to marginal granules	Flagellum	Greatest width	Total length	Range in total length
1	8.6	9.1	17.7	37.4	1.8	55.1	45.4-58.3
2	8.2	8.6	16.8	29.0	2.0	45.8	38.2-49.5
3	6.4	7.7	14.1	34.3	1.8	48.4	36.0-54.0
4	6.8	8.5	15.3	22.7	2.0	38.0	31.0-40.5
5	4.5	7.3	11.8	21.8	1.8	33.6	27.0-36.5
Average	6.9	8.2	15.2	29.0	1.9	44.2	

The accompanying figure (Text fig. B) represents diagrammatically the cell structure and the terminology applied to the various parts of the cell. There is a rigid cell body from the anterior end of which emerges a flagellum, or much more frequently the two flagella of the dividing individual, enclosed in a cytoplasmic sheath. Wenyon (1913) considers that the body of the cell is much flattened like a blade of grass. In the average stained preparation where one gets only a side view of the flagellate this appears to be true; but in order to determine whether this was actually the case, I examined a peritrophic membrane filled with the flagellates, many of which had attached themselves by their flagella in such a position that their longitudinal axes were parallel to the rays of light passing through the microscope. By focusing up and down upon such individual flagellates it was apparent that their optical cross sections were round or oval. The entire cell is enclosed in a thin periplast within which lies the endoplasm and the cell organelles, which will be described separately.

In comparison with *Crithidia gerridis* (see Becker, 1923) the endoplasm is finely granuled. The "chromidia" lying in the endoplasm

are smaller than those in *Crithidia gerridis,* and do not stain *intra vitam* with Janus green. Swellengrebel (1911) reported that these "chromidia" are composed of volutin. Wenyon found bodies lying in the endoplasm which he thought were bacteria because of their bipolar staining. I have not found these appearances with the frequency with which Wenyon found them, but at times I have found rod-like nuclear fragments in what appear to be degenerating specimens with disrupted nuclei. They stain much heavier, however, than the bacteria on the slide, and are probably not the bodies which Wenyon considered bacteria. In my preparations I can find no evidence of the presence of bacteria in the endoplasm of the cell.

This brings us to the question of a cytostome, which Wenyon (1913) has discussed at length. I have carefully searched in the living and stained flagellates for the funnel-like depression surrounding the flagellum which Wenyon describes, but I can find no evidence for it. It is true that the region of the cell body anterior to the parabasal body



Text fig. B.—Diagrammatic representation of *Herpetomonas muscae-domesticae; f,* flagella; *m,* marginal granules; *r,* intracytoplasmic flagellum or rhizostyle; *b,* basal granules; *p,* parabasal body; *s,* periplast; *e,* endoplasm; *n,* nucleus with nuclear membrane and central karyosome; *c,* chromidia.

is often remarkably transparent, particularly between the two flagella of a dividing cell; but there is no visible aperture through which solid particles of food such as bacteria could be admitted. The periplast enclosing the cell body is in uninterrupted continuity with the flagellar sheath so far as one is able to observe. The anterior end of the cell body of the flagellate with which I worked is not truncated after the fashion of Wenyon's figures. The absence of solid food particles in the endoplasm and the absence of any cytostome suggest that the parasite is saprozoic in its habits.

Prowazek (1904) described the organism as a biflagellate. Almost all subsequent observers (Patton, Porter, Wenyon, Mackinnon, Strickland, etc.) have agreed that the biflagellate appearance is due to the tendency of this organism to be almost constantly in a stage of division, since under some conditions both uniflagellated and biflagellated specimens can be found. The cyst normally contains but the rudiment of one flagellum (Fig. 19) and the trypaniform type (Figs. 17, 18) in most instances is uniflagellated, although dividing specimens with two flagella exist.

The flagellum arises from the basal granule and proceeds anteriorly as a fine line until it reaches the margin of the cell, where it thickens noticeably to form the marginal granule; or in the dividing forms, the marginal granules (Swellengrebel, 1911) (Figs. 3, 4, 5) which Prowazek termed the (anterior) diplosome. The intracellular portion of the flagellum has often been termed the rhizoplast (Prowazek, Swellengrebel). The extracellular portion of the flagellum is much more sturdy and takes a deeper stain than the intracellular portion.

The basal granules lie immediately anterior to the parabasal body (Figs. 2, 4, 7, 9, etc.) sometimes in contact with it (Fig. 13). Sometimes the intracytoplasmic portion of the flagellum can be traced until it makes a contact with the parabasal body, with no basal granules visible (Figs. 3, 6, 8). In such cases the basal granules must lie either embedded within the parabasal body or in a depression in its anterior surface.

The parabasal body (blepharoplast of Schaudinn, kinetonucleus of Woodcock) in the adult herpetomonad lies usually from 3.5 to 4.5μ anterior to the nucleus. It varies somewhat in shape from pear-shaped to bar-shaped. In the dividing specimens it may appear heart-shaped (Fig. 9). In the living cell it is seen with difficulty as a more or less refractile spot. If an infected intestine is teased up in a drop of Janus green made by dissolving one part of the powdered dye in twenty thousand parts of normal saline solution, the parabasal bodies of the flagellates stain after the typical mitochondrial fashion, which the nucleus does not do. In dried preparations stained by Wright's stain this structure appears wedge-shaped, and sometimes quite elongated as indicated in figure 20. This distortion is due to the drying process. A discussion of the parabasal body of these flagellates was included in a previous paper on *Crithidia gerridis*.

No investigator has considered the cytological details of the nucleus so thoroughly as has Wenyon (1913). He describes the nucleus thus: "There is a fine nuclear membrane enclosing a space, in the center of which lies the deeply staining karyosome." In addition there was observed what the author conceived to be a centriole, which may be within the center of the karyosome (composed of chromatin and plastin), just outside the karyosome, or situated on the nuclear membrane. My own observations agree in the main with those of Wenyon, except in regard to the existence of a visible centriole. It is not to be questioned that chromatin granules, which resemble centrioles, may be found in the nucleus, but after studying a large number of dividing specimens I have became convinced that there is no indication of a centriole or other attraction body which performs the function of a centriole during the process of division. Alexeieff (1912) who studied

carefully the process of nuclear division in this parasite found no indication of a functioning centriole. The nucleus will be discussed further under the process of division.

Alexeieff describes a rhizostyle or axostyle extending posteriorly from the parabasal body of the hemoflagellates, which is evidenced by either positive or negative staining. Like Wenyon, I am unable to find anything in well-stained preparations like the appearances which Alexeieff figures, but occasionally near the edge of the smear where the specimens have dried somewhat, the dark staining granules in the cytoplasm may accidently be arranged in rows due to endoplasmic streaming, which was not observed in this species of flagellate, but was observed in *Crithidia gerridis.*

In addition to the adult herpetomonads just described, there are two other stages of this flagellate commonly present, which will be described separately, the trypaniform and the cyst.

The trypaniform stage may be regarded as the transitional stage between the adult flagellate and the cyst form. It is found usually in the posterior part of the midgut and in the rectum. Wenyon has described two methods of encystment, one in which encystment is preceded by the trypaniform stage and one in which it is preceded by the leptomonas or herpetomonad stage. This is quite markedly true in the case of the small flagellates which I found in *Muscina stabulans,* but in *H. muscae-domesticae* I have observed only one manner of encystment, and that from the trypaniform type, which in turn is derived from the adult herpetomonad type. All intermediate stages may be found from that in which the parabasal body approaches the nucleus and becomes in a sense a crithidia (Fig. 16) to the stage where the parabasal body lies posterior to the nucleus and is structurally a trypanosome, although in neither the crithidial nor trypanosome stage is there an undulating membrane. The body decreases noticeably in length and very slightly in width as the trypaniform condition develops. Usually one flagellum and one nucleus are present, but occasionally dividing forms with a double set of organelles are found.

The parabasal body of the trypaniform type continues to migrate posteriorly, and the cell body becomes shorter, until the organism becomes a small oval body with the mere rudiment of a flagellum (Fig. 18). The extracellular flagellum finally degenerates completely (Fig. 19). The point of entrance of the flagellum into the cyst is plugged by the marginal granule, which continues to stain deeply. The intracellular flagellum can be traced to the parabasal body which finally migrates to the extreme posterior end of the cyst, becomes bar-shaped, and stains less deeply than formerly. The nuclear karyosome becomes round or oval, and lies inside a light staining nuclear membrane. The protoplasm

nearest the flagellum stains somewhat more lightly than that near the edge of the cyst, but why Strickland (1911) should call this pale staining endoplasm a cytopharynx is not clear.

METHOD OF MULTIPLICATION

The only method of multiplication observed in this protozoon is binary fission. The processes of copulation, parthenogenesis, etheogenesis, and autogamy, as described by Prowazek, are now known to have been based upon misinterpretations. As Kofoid (1916) has said, ". . . we have as yet, except among the Volvocidae, which are hardly typical flagellates, little evidence which can be regarded as final that true sexual reproduction occurs at all among flagellates."

Wenyon (1913) and Alexeieff (1912, 1913) made important studies on the process of division of this flagellate, particularly in regard to the nucleus. In my slides fixed in Schaudinn's solution without the acetic acid and stained by the iron-hematoxylin method I have frequently found beautifully stained dividing specimens on which I made certain observations agreeing except in a few details with those of Alexeieff, but differing somewhat from those of Wenyon, particularly in regard to the presence of a functioning centriole.

The first indication of division is a divided basal granule and a double flagellum (Figs. 3, 4, 5, 6). By far the greater number of flagellates exist in this dividing stage. The doubling of the flagellum comes about, according to Wenyon, Patton, Mackinnon and others, through a new flagellum growing out on intimate contact with the old one, which makes the process difficult to observe. As the two basal granules move away from each other, the parabasal body enlarges somewhat, and commences at the anterior end to split longitudinally (Fig. 9). At this stage it is usually heart-shaped. The splitting continues under the influence of the basal granules until the new pear-shaped parabasal bodies are entirely separate (Figs. 10, 11). There is no evidence of a mitotic division of the parabasal body such as Rosenbusch (1909) described for trypanosomes and Whitmore (1911) described for *Prowazekia asiatica*.

The apparent non-uniformity of the appearances in various dividing nuclei is somewhat discouraging to one who would get such clear-cut pictures of the various stages as are possible in metazoan cells. The difficulties are somewhat increased if one postulates the presence of a centriole, as Wenyon did, and attempts to follow the division in relation to these chromatin fragments resembling centrioles. Wenyon himself expressed the opinion that many of the appearances which he considered centrioles may have been simply separate chromatin bodies. It seems to me that the latter possibility is the correct one; and like Alexeieff,

I should place this type of nuclear division in the group with those which have no functioning centriole. Alexeieff calls this type of nuclear division panmitosis, and defines it thus:

"Tout le matériel chromatique (chromatine périphérique ainsi que chromatine caryosomienne) est employé à la formation des chromosomes, de sorte qu'il n'y a ni centrioles ni corps polaires. Le stade de la plaque équatoriale n'est pas net, même le plus souvent il manque complètement. A l'anaphase entre les deux noyaux-fils est étirée une fibre fusoriale."

The resting nucleus may be regarded as a plastin matrix surrounded by a fine achromatinic nuclear membrane in whose center is embedded the karyosome, composed either of one or several chromatin particles (Figs. 2, 3, 4, 21-25). As was previously noted by Mackinnon (1910), one of the first indications of division in the nucleus is elongation of the karyosome in the longitudinal axis of the cell (Figs. 26-29). As the cell body increases in breadth the elongated nucleus is drawn to a position at an angle to its former one (Fig. 30). By this time the nuclear membrane has become indistinguishable from the cytoplasm, and the karyosome begins to resolve itself into a number of discrete granules (Figs 7-10, 31-33). The chromatin mass becomes constricted in the center as the two halves move away from each other. Alexeieff states that the nucleus may be divided into from eight to sixteen chromosomes, but he figures four in each daughter nucleus. I have failed to find clearly more than four of these chromatin fragments or "chromosomes" in each of the daughter nuclei. In some cases where there are two or three granules one or two of them are usually exceptionally large or of a bilobed appearance, suggesting that they are in reality double.

The two daughter nuclei may be connected by an achromatinic filament (Fig. 9), or there may be none present (Figs. 10, 11). This is Wenyon's centrodesmose and Alexeieff's *tractus fusorial achromatique*. It stains somewhat lighter than nuclear chromatin, and probably represents the interzonal nuclear plastin undergoing degenerative changes prior to absorption into the cytoplasm.

The cytoplasm of the mother cell splits longitudinally to the posterior end (Fig. 13) and the two individuals remain connected only by the sheath (Fig. 14). Very soon they free themselves from each other by their active movements. The chromatin fragments in the nucleus again clump together to form the resting nucleus.

LIFE HISTORY STUDIES

In his various papers upon Crithidia and Herpetomonas Patton has always emphasized that these flagellates in their life cycle pass through pre-flagellate, flagellate, and post-flagellate stages. It is perhaps due to

his influence that it is to be inferred from almost all the publications upon these subjects that this is an obligatory life cycle and that the cyst is the only infective stage. For example, Strickland (1911) and Wenyon (1913) speak of the cyst as the infective form of *H. muscae-domesticae*. Roubaud (1912) did a series of experiments with Pycnosoma flies and their flagellate parasites from which he concluded that the infection is transmitted from fly to fly by the cysts and not by the flagellated forms found in the feces.

Patton himself (1910) performed certain experiments with *H. muscae-domesticae* in the host *Musca nebulo*, which convinced him that the flagellated form as well as the cyst was infective. He found that if he fed "clean" flies of the species *Musca nebulo* upon spleen where infected flies had previously deposited their excreta, and that if he examined the intestinal contents of these clean flies within three hours from the time they were allowed to feed, flagellated herpetomonads would be present. On the second day he found numerous dividing forms. The infections produced in this manner were never so heavy as those found in "wild" flies.

Before I learned of Patton's work, I had carried out certain experiments with *H. muscae-domesticae* in the host *Phormia regina* which seemed to demonstrate conclusively that the flagellated forms may transmit the infection under certain conditions. Because a different method of attacking the problem was employed and because my results amplify as well as confirm Patton's work, it seems well to publish them.

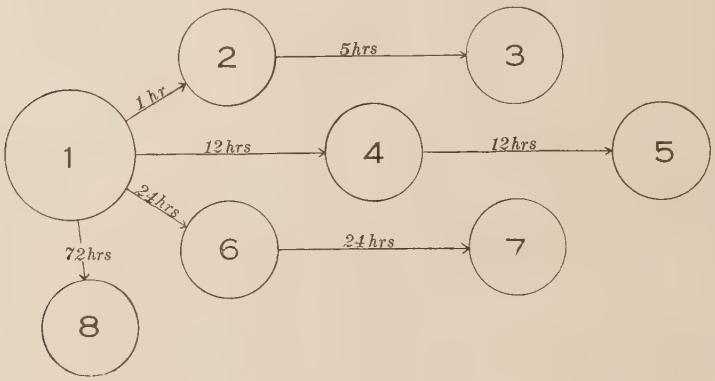
"Clean" (uninfected) flies were raised in the laboratory by the following method. A number of adult flies were put in a covered jar with a piece of rat flesh or ground beef. After the female flies had deposited their eggs upon the meat, it was transferred to another jar with an inch or two of sawdust on the bottom and covered with cloth. The young larvae fed upon the meat until they reached the adult larval stage, when they migrated down into the sawdust and pupated. Very soon after hatching the flies were etherized or chloroformed and distributed among jars covered with cheese-cloth. The food of the fly consisted of only a slightly alkaline (P_H 8) dilute sugar solution. The sugar solution, jars, and cheese-cloth were boiled before using and while in use were kept inside a bookcase to prevent infected flies from depositing their feces upon the covers of the jars.

The "clean" flies were distributed so that one container held about thirty flies and seven others twelve flies each. They were fed sugar solution shortly after hatching, and then starved for twenty-four hours. At the end of that time a number of wild flies were examined microscopically for parasites, and the anterior ends of several heavily infected intestines were teased up in 0.4 per cent. NaCl solution and fed to the

flies in the jar containing thirty flies, designated as jar 1. Most of the flies fed upon the infected material, and within a few minutes all the liquid had either been eaten by the flies or had dried up..

At the end of intervals of one, twelve, twenty-four, and seventy-two hours from the time of feeding the infected liquid, flies were removed from jar 1 and examined for flagellated forms. Upon each examination flies were found which still held in their intestines some of the flagellates which they had ingested at the time of feeding the infected material. The intestinal contents of such flies was at once fed to clean flies in other jars, designated as jars 2, 4, 6 and 8 (see text fig. C).

Five hours after feeding, several of the flies in jar 2 were examined, and the flagellate stage was found to be present in the intestines of some of them. Immediately the infected intestinal contents was fed to flies in jar 3. In like manner some of the flies in jars 4 and 6 were



Text fig. C.—Plan used in fly infection experiment; circles represent glass jars containing flies; lines, time intervals between feeding of intestinal contents of infected flies from containers to left to flies in containers to right; further details in text.

examined for flagellates at respective intervals of 12 and 24 hours from the time of the infective feeding, and the intestinal contents from each was fed to the clean flies in another container (jars 5 and 7). Further, flies were removed from jar 1 at periods of 96, 120, 144, and 168 hours, and 15 days after the original infective feeding.

The examination of flies from jar 1 at intervals of 1, 12, 24, 72, 96, 120, 144, 168 hours, and 15 days from the time of attempted infection proved that the ingested flagellates and the flagellates which arise from them by binary fission may be found in the intestine after these periods of time. At intervals of ten days from the time they fed upon the infected material, the flies in jars 2, 3, 4, 5, 6, 7 and 8 were examined microscopically for parasites. From one-third to three-fourths of the flies which remained alive in each jar carried infections, most of them

fairly heavy. The tremendous increase in numbers showed that the flagellated forms originally ingested by the flies had succeeded in establishing themselves and multiplying in the flies' intestines.

There was a two-fold reason for passing the parasites through two and three hosts. First, if there had by chance been any cysts in the original infective material which was taken from the anterior ends of the alimentary tract of infected "wild" flies, the various time intervals would have given them time either to excyst or to be carried passively through the fly's intestine. Second, it proved that there is no definite physiological cycle which must be undergone before a Herpetomonas ingested by one host is infective to another. It will be remembered that Minchin and Thompson (1915) found that within one-half hour after a rat flea has ingested the blood of a rat infected with *Trypanosoma lewisi,* a physiological change occurs which makes the trypanosome incapable of infecting another rat when injected into it, until after a developmental process requiring at least five days has taken place. These fly experiments indicate that there is no comparable obligatory cycle in *H. muscae-domesticae* which must take place before one *Phormia regina* can be infected from another.

Proper controls of non-infected flies were examined throughout the experiment. Ten flies hatched at the same time as those used in the experiments were examined for parasites at the beginning of the experiment and were found to be negative. Fifteen others were kept in a separate container and ten days after hatching were found to be negative.

Fly experiments of this nature would be of little value if the infection could be transmitted "hereditarily" through the ovum. Prowazek (1904) claims to have demonstrated that the ova of *Sarcophaga haemorrhoidalis* Fall may be invaded by Herpetomonas, where they undergo a developmental process and infect some of the larvae. Since Prowazek's observation attempts to transmit the infection "hereditarily" have met with failure. Roubaud (1912) working with *Pycnosoma putorium* and Patton (1910) working with *Musca nebulo* were unable to demonstrate hereditary transmission of Herpetomonas. Patton states, "My experiments have clearly demonstrated that *Herpetomonas muscae-domesticae* is not transmitted through the egg of *Musca nebulo.*"

In my various experiments with various species of diptera, I have employed more than six hundred flies, largely bred from infected wild flies. About 140 of these flies were examined at various intervals after hatching for control purposes. In no case have I ever found so much as one control fly infected. These flies belonged to the species mentioned in the first part of this paper. The two following experiments were done with Sarcophaga.

A female *Sarcophaga bullata* captured in August carried the heaviest infection with *Herpetomonas muscae-domesticae* which I have ever found in a fly. The abdomen carried a number of larvae which were placed on a piece of fresh meat and from them about twenty-five adult flies were raised. Ten of these flies were examined at once after hatching and fifteen others after an interval of five days. Herpetomonas was not found to be present in the intestines.

A female *Sarcophaga securifera* captured the same month was found to have a fairly heavy infection with *Herpetomonas muscae-domesticae*. Its brood was kept under the same conditions as that of *S. bullata;* of the twenty-one flies which hatched, none were infected after two days.

It must be admitted that neither my results nor those of other workers prove conclusively that hereditary infection with Herpetomonas may not occur exceedingly rarely in muscoid flies. At any rate the evidence for it is very meagre, and it is impossible to accept this part of Prowazek's work until more positive experimental evidence is produced.

The exact status of larval infections is somewhat complicated. Prowazek (1904) found infected Sarcophaga larvae. Patton (1910) reported that the full grown larvae of *Musca nebulo* were not infected, but that he had found a few long flagellates in very young larvae which had fed upon the bodies of dead infected flies. At that time he reported that the full-grown larvae, pupae, and adults bred from such larvae were uninfected. More recently (1921) the same author reports finding larval infections, and that the infection is carried thru the pupal stage to the adult. Mackinnon (1910) reported larval infections in the case of Scatophaga and Homolomyia, but was unable to find infected larvae of *Musca domestica*. Strickland (1911) was unable to find infected Lucilia larvae.

During the months of July and August, 1922, I examined two hundred fly larvae, principally Lucilia and Phormia, in various stages of development, taken from the most exposed places in Westport dump (Baltimore). No infected larvae could be found. I also examined sixty *Musca domestica* larvae taken from a pile of horse manure, but I found no infections. An attempt was made to experimentally infect the larvae of *Sarcophaga bullata*, the adults of which are parasitized to the extent of about fifteen per cent. in nature. A small amount of ground beef was thoroughly mixed with the infected intestines of about twenty-five Phormia flies. The flagellates could easily be found microscopically in the liquid from the beef. Some of this material was fed to ten uninfected adult flies *(Sarcophaga bullata)*. Ten days later three of them were found to be infected. Thus the ground beef was proved to be infective. About fifty young larvae were placed upon this beef,

and then left there for twenty-four hours. At the end of forty-eight hours an examination of the intestines of ten larvae gave negative results. Examinations of the larvae made each succeeding day for one week were also negative.

It is difficult to draw any conclusions from the conflicting observations of various authors, but it seems that one of two factors must be at work here. It is possible that the larvae of certain species of muscoid flies are capable of infection with herpetomonas, and that certain others are not. Then there is the other possibility that certain environmental conditions, relating to temperature, moisture, character of food, flora of the larval intestine, etc. are factors which determine whether or not larvae can be infected. Much more work needs to be done along this line in order to determine what are the factors which determine the presence or absence of infections in fly larvae.

NOMENCLATURE *

There has been much controversy concerning the generic names Leptomonas and Herpetomonas. Kent (1881) gave the former name to a flagellate from a free-living nematode, *Trilobus gracilis*, and the latter to the flagellate from the house-fly, the descriptions being so similar that it is impossible to make any important distinction between the two. The fact that the first reviser, Bütschli (1884), united the genera Hepetomonas and Leptomonas and selected the former as the name for the composite genus, and the further fact that the type species of Herpetomonas is easily obtainable, while the type species of Leptomonas is not, makes it obligatory that the name Herpetomonas be accepted and Leptomonas rejected. (International Rules of Zoological Nomenclature, Article 28.) This decision is necessary in spite of the fact that in Kent's original work Leptomonas has page priority over Herpetomonas.

The specific name *muscae-domesticae* was originally ascribed to Burnett by Stein (1878), and later ascribed to him by Kent (1881), Hindle (1912), and other writers subsequent to Stein. In spite of the fact that I have carefully read all the references to Burnett given by Stein, Kent, and Hindle, as well as a number of other papers by Burnett, I am unable to find that he ever referred to this species other than as "*Bodo* of the common house-fly." The name *muscae-domesticae* has, therefore, to be referred to Stein, although previous to Stein's work Leidy had proposed the name *Bodo muscarum* for a "protohelminth" from the fly *Musca domestica*. In the absence of either a recognizable description of the parasite or a reference to

* The main conclusions regarding nomenclature were reached after communication with Dr. C. W. Stiles, to whom I am very much indebted for his opinion on the subject.

Burnett's description we must consider *Bodo muscarum* a *nomen nudum*. The correct name of this protozoon is, therefore, *Herpetomonas muscae-domesticae* (Stein, 1878, nec Burnett) Kent, 1881.

Chief synonyms of the common flagellate entozoic in *Musca domestica* are

Bodo of the common house-fly, Burnett, 1852.
Bodo muscarum Leidy, 1856, nomen nudum, does not mention Burnett.
Bodo muscae domesticae Burnett of Stein, 1878.
Cercomonas muscae-domesticae (Stein, 1878) Stein, 1878.
Cercomonas muscae Leuckart, 1879.
Leptomonas muscae-domesticae, Dunkerly, 1911.

CONCLUSIONS

1. *Herpetomonas muscae-domesticae* was found to be entozoic in the North American muscoid flies, *Musca domestica, Phormia regina, Lucilia sericata, Calliphora erythrocephala, Cochliomyia macellaria,* and *Sarcophaga bullata.*

2. The seat of infection is throughout the length of the alimentary canal.

3. The flagellate in its life history exhibits the adult long flagellated form, the cyst, and the intermediate stages from the crithidial to the trypaniform type.

4. The parabasal body stains with Janus green after the typical mitochondrial fashion.

5. The only method of multiplication found was binary fission. During division the chromatin of the nucleus is resolved into a number of fragments, usually four in each of the daughter cells.

6. Feeding experiments indicated that the flagellated form of the parasite is infective, and that there is no obligatory cycle which must be completed before the parasites of one host are infective to another.

7. No evidence for hereditary transmission of *Herpetomonas muscae-domesticae* was found.

8. Attempts to find infected larvae or to experimentally infect them were failures.

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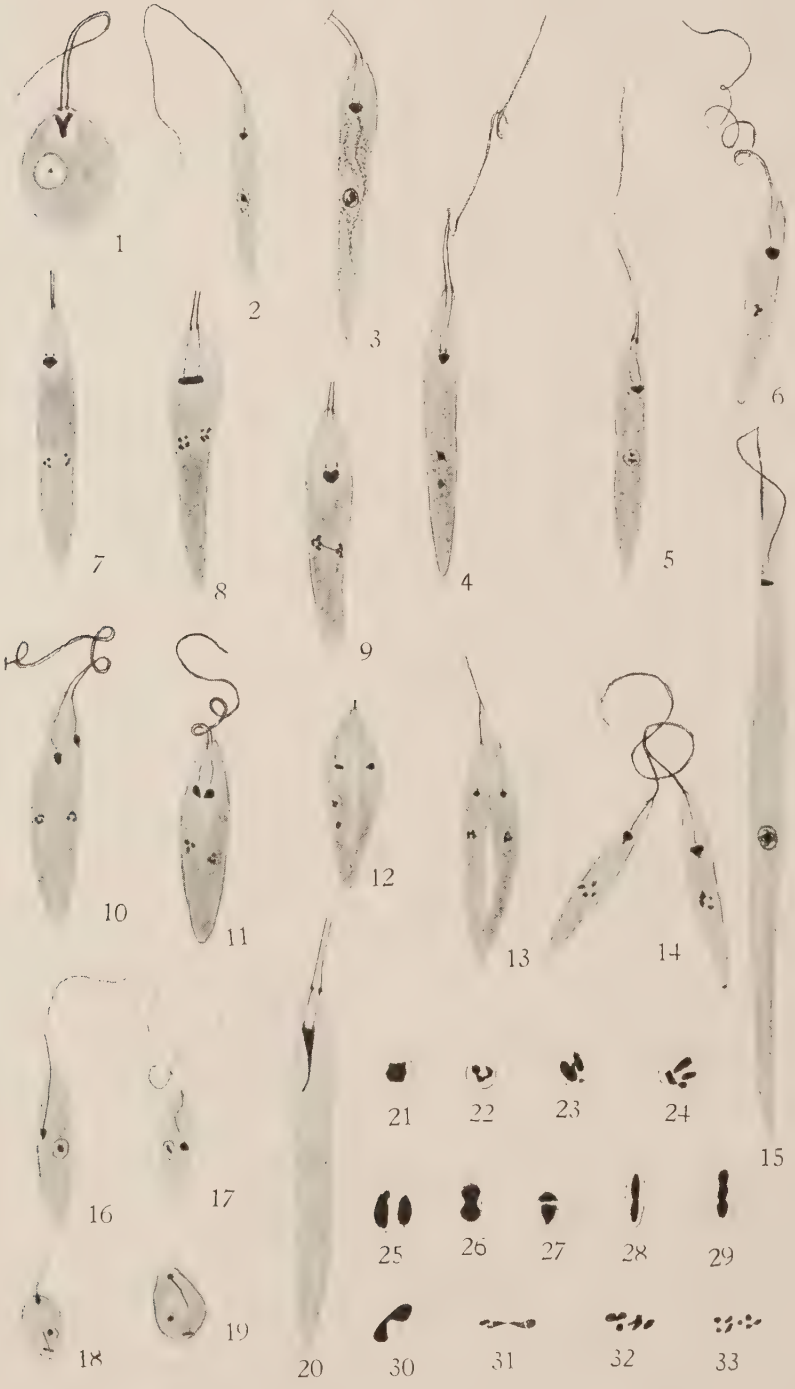
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PLATE XXI

Patton, W. S. 1910.—Experimental Infection of the Madras Bazaar Fly, *Musca nebulo Fabr.* with *Herpetomonas muscae-domesticae* Burnett. Bull. soc. path. exot., 3: 264-273.

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Explanation of Plate XXI

Camera lucida drawings; × 2140.

1. Herpetomonas found in *Sarcophaga securifera.*

2. Adult uniflagellated *H. muscae-domesticae.*

3. Biflagellated individual with accidental arrangement of cytoplasmic granules posteriorly from parabasal body simulating rhizostyle.

4-6. Forms with the double flagellum.

7. Division stage with two and three chromosomes in daughter nuclei.

8. Four chromosomes distinctly visible in each daughter nucleus.

9. Parabasal body in process of dividing. A chromatinic filament joins daughter nuclei.

10-11. Other stages in division of parabasal body.

12. Abnormal condition in advanced stage of division where both daughter nuclei remain in the same cell, although cytoplasm of parent cell has commenced to divide to form daughter cells.

13-14. Daughter flagellates have almost completed division process; nuclear chromatin still remains fragmented.

15. A "monster."

16. Parabasal body has commenced to migrate posteriad. Proximity of parabasal body to nucleus makes this a crithidial stage.

17. Parabasal body has migrated further posteriad, and cell has shortened noticeably. Very often in this stage parabasal body is posterior to nucleus.

18. Extremely small trypaniform type about to lose flagellum to become cyst.

19. Large cyst.

20. Dried flagellate stained by Wright method. Parabasal body stains deep magenta; nucleus, pink.

21-33. Stages in nuclear divisions not according to scale.

21-25. Resting nuclei.

26-29. Elongation of nucleus, first indication of division.

30. Nucleus drawn to an angle with longitudinal axis. Very often the chromatin at this stage is fragmented.

31-33. Just prior to constriction into two daughter nuclei.

33. Nuclear chromatin has resolved itself into eight discrete granules, four of which will probably go with each daughter cell.

NOTES ON THE OCCURRENCE OF *TAENIA SAGINATA* IN NORTH CHINA*

RALPH G. MILLS

In a recent communication from this department Faust (1923) says "Twenty-five years ago infestment with *Taenia saginata* was common in North China. The infection was brought down in cattle from beyond the Great Wall, which were slaughtered immediately and offered for sale in the markets. Today such infection occurs rarely in Peking and vicinity. The cattle come from the same locality and are presumably infected, but for economic reasons they are fattened for a period of from several months to a year in local yards and, when slaughtered, are relatively free from infection." The impression is quite general that the beef obtainable in Peking is not infected with tape-worm and is therefore safe for consumption without special inspection. The fact that this is an erroneous assumption is the primary reason for recording the following observations.

Through the cooperation of an intelligent German butcher I have secured numerous samples of beef containing *Cysticercus bovis*. One piece weighing 3 lbs. from the leg muscles of an ox contained 4 cysts, and another weighing 5 lbs. contained 10. Four of the animals were bulls and one a cow in whose tenderloin several cysts were found. By casual examination then he discovered 5 infected animals in less than 300 examined, or roughly 2 per cent.

Careful routine examinations made for municipal and scientific purposes by Hertwig (cited by Fantham, Stephens and Theobald, 1916) showed "that the cysticercus of the ox is found chiefly in the musculi pterygoides externi and interni, and since that time a far greater number of infected oxen have been found in Berlin." If, then, the infection in the leg muscles reaches the extent observed it is highly probable that a systematic search of the tongue and throat muscles would yield a much higher per cent. of infection, as it did in Germany. The investigations of Hertwig lead to certain municipal regulations which are summarized by Fantham, Stephens and Theobald thus, "The flesh of oxen only slightly infected (containing not more than 10 living cysticerci) is sold in pieces of not more than 5 lbs. to customers after having been rendered innocuous by cooking, or by pickling for 21 days in 25 per cent. salt brine, or hanging for 21 days in suitable refrigerators; oxen in which only one cysticercus is found are allowed free commerce, and those strongly infected (i. e., containing more

* From the Department of Pathology, Peking Union Medical College.

than 10 living cysticerci) may only be used for industrial purposes." Reissmann (quoted by Fantham, Stephens and Theobald) states that more bulls than cows are infected, 0.446 per cent. for bulls and 0.262 per cent. for cows, a condition explained by Ostertag by the fact "that most oxen are killed when young, when also infection most readily takes place, and further, that the larva later on in life can be completely atrophied."

On the basis of unofficial statements from the butcher and a collector of the tax on imported cattle, it appears that about 15,000 animals are brought annually to this vicinity. They are nearly all bulls between 6 and 9 years old, and a few older cows. Approximately one third are shipped as dressed meat to the north city, another third are consumed in the south city, and the remainder are for the extra-mural population. The annual consumption of beef by those living within the walls is about 10,000 animals, or one beef for 80 persons. The proportion for the ordinary Chinese population is actually very much less when deductions are made for the number consumed by 3,000 foreigners, numerous wealthy Chinese and the large number of restaurants and hotels. The poorer people rarely eat beef, as mutton and pork are much less expensive. Of the total number of cattle, roughly two-thirds come from the region about Kalgan, the remainder being raised (or possibly temporarily fed) in the vicinity of Peking, and a very few brought in from Shantung Province. A few milch cows are also imported by local dairies from Japan or other parts of China.

As a matter of some interest, the cases of tapeworm infection that have been admitted to the Peking Union Medical College during the past two years were examined with the following results:

Hospital Number	Age	Sex	Race	Occupation	Residence	Diagnosis of		
						Taenia saginata	"Tape-worm"	Ova in Stools
235	21	M.	Chinese	Weaver	Peking	..	—	..
266	21	M.	Chinese	Student	Peking	..	..	—
1345	24	F.	U. S. A.	Secretary	Peking*	—	..	..
2311	22	F.	Chinese	H. W.	Peking	—	..	..
2494	32	M.	Chinese	Preacher	Peking	..	—	..
2960	18	M.	Chinese	Student	Peking	—	..	..
2974	19	M.	Chinese	Student	Honan	..	..	—
3297	22	M.	Chinese	Telegrapher	Peking	..	—	..
3815	14	M.	French	Mechanic	Peking†	—	..	..
4269	28	M.	Korean	Student	Peking	—	..	..
4353	22	M.	Chinese	Student	Shansi‡	..	..	—
4373	19	M.	Chinese	Student	Peking	..	—	..

* In Japan 1 year, in Peking 6 months.
† Came from Siberia 2 years ago but segments seen for 1 month.
‡ In Peking 18 months.

The above cases include all hospital patients infected with any form of tapeworm in the adult state except one who harboured *Hymenolepis nana*. No case of *Taenia solium* infection was observed. So far as

could be determined, only one of the patients came from north of Peking. Several patients state that they had tried various native and Japaneses remedies before applying to the hospital for treatment. In most instances the patients knew of their condition, the exceptions being those in whom the ova were found by accident in the stools. These did not remain for treatment and the diagnosis is therefore left in doubt. The Chinese woman admitted to the obstetrical ward had been "needled" by a native doctor in the treatment of the abdominal distension which had been attributed to the presence of a tapeworm. The patients in whom only "tapeworm" was diagnosed were those in whom the specimen was not recovered or where treatment was not administered for various reasons. There was no doubt as to the presence of a tapeworm, and it may with certainty be assumed to be *T. saginata.* A glance at the occupation of these cases indicates the high average of intelligence of practically every patient. This may indicate the class of people that seek hospital treatment, rather than any selective incidence of the disease. The reliance upon native remedies alone would be found more among the more ignorant classes of people and the occasional success of such measures would undoubtedly cause the incidence of the disease to appear to be much less than it really is.

Perroncito (cited by Fantham, Stephens and Theobald) found that the adult tapeworm grows about 3 inches per day. At this rate the ripe segments would certainly appear in the stools within two months after the infection occurred. This fact would be of value in deciding when and where the infection probably took place. In this series the Frenchman came from Siberia more than a year before, the American Secretary arrived in Peking at least 5 months before segments were seen, the Chinese student from Honan had been in Peking several months before noticing the infection, the Shansi student arrived 18 months previous, and the Korean student had lived for several years in China. We are, therefore, safe in concluding that all the cases represent local infections. In addition to those patients mentioned are a number of foreigners, mostly British and Americans, who have been treated for tapeworm infection by various physicians of our own staff and those practicing in the city. The number of known cases is about equally divided between Chinese and foreigners.

Mention was made above that animals kept in and about Peking were not so liable to harbour the infection as those coming directly from Mongolia. Where cattle are herded on the open plains the opportunities for them to eat grass contaminated with human feces containing viable tapeworm eggs would be great. The opposite condition obtains in most other places in China where animals are separated

and kept in barns or small enclosures where prepared food is given to them. The chances of widespread dissemination of such infections would be slight and this doubtless operates in an effective way in eradicating any sporadic outbreak. There are apparently no reports of the finding of cysticerci in beef from any place in China. Cases of tapeworm in man are not uncommon and doubtless have not been considered worth reporting for that reason. Maxwell (1921) reporting on intestinal parasitism in South Fukien, mentions only one case of *T. saginata* in man. This patient had been in the Straits Settlements and while there was treated for tapeworm. Upon his return he was again treated, presumably for a recurrence of the same infection. Examination of samples of beef disclosed no evidence of cysts. Pork was also free from similar infection and no cases of infestation with *T. solium* were observed.

Cooks, butchers, and others who handle or prepare foods are found to harbour the parasites in higher percentage than those engaged in all other occupations. The small series cited contains only one person, the housewife, who had any connection whatever with food in preparation. It is interesting to note that among the hundreds of house servants examined here in a special clinic for the purpose, few cases of tapeworm infection were found. In 1920 Korns reported the results of the examination of 400 domestic servants, 328 men and 72 women, employed by foreigners, representing 88 households. The ova of the tapeworm was found in the stool of one individual. In another series, published in 1921, including 672 men and 128 women from 145 households, no cases of infection by tapeworm were observed. Ernest Tso (1923), in a report in press, summarizes the findings in 950 employees of the Peking Union Medical College, in 132 of whom the stools were examined without detecting the presence of any case of tapeworm. In addition, there were perhaps 100 domestic servants examined in the same clinic, which is a continuation of that conducted by Dr. Korns, without discovering any further case. Mr. Cameron, pharmacist to the Peking Union Medical College, informs me that he has filled only a few prescriptions for tapeworm treatment for persons in the outpatient department and that this drug is supplied to the hospital wards on an average of about once a month. This agrees very well with the series of cases from the wards cited above, the medicine for whose treatment was supplied from the drug room.

My office secretary was commissioned to secure what information he could from native sources. Most of the medicine for the treatment of tapeworm is purchased on the street or in the fairs from itinerant drug merchants and relatively little is bought in the established native drug shops. A good deal is sold, thus indicating that the infection is relatively common. This medicine is not regarded as being very

efficacious and some cases that he came across had tried repeatedly to dislodge the worm without success. One fat, healthy man whom he met said he had carried his tapeworm for twenty years and had given up all efforts to rid himself of it. Another case was that of a cook in a British family who had evidently evaded examination because he believed that the hospital treatment for tapeworm involved the use of the knife. From this it would appear that the infection is much more prevalent than some of the above mentioned investigations would lead us to believe.

As to prophylaxis, the municipal regulations mentioned above serve as a safe guide. Refrigeration is safe if continued long enough, but facilities are almost wholly lacking in the Orient. There is no information at hand to indicate whether or not the drying of beef kills the cysts. However, dried beef is cut so thin that by ordinary inspection of the slices any cysts present could be readily seen. Boiling or thorough roasting would, of course, render the meat harmless. The difficulty in this process is that the center of a large piece of meat does not reach the same temperature as the outside. The degree of heating can only be judged by the appearance of the meat. Perroncito found by feeding experiments on calves that 45 C. was the lethal temperature for the cysts and he put the matter to a test on a number of people. Lower temperatures had little or no effect. The protein of beef coagulates at 55 to 66 C. and hence such a temperature would render the meat perfectly safe. This does not completely remove the reddish color, but each muscle fiber has been changed from a semi-translucent to a perfectly opaque structure. It may, therefore, be confidently assumed that unless this change has taken place, the meat may still contain viable cysts. Inquiry among the Chinese in Peking as to the ways of preparing beef indicated that in most instances the meat is boiled. Another method is to soak the flesh in brine and then allow it to dry, eating it perhaps weeks or months later. Meat and vegetables are also mixed together in a finely chopped condition, wrapped in a sheet of dough and then steamed. Opinions seemed to differ as to whether the meat was previously cooked, and, if so, how much. Beef was apparently never eaten in the raw or unprepared condition. Beef and vegetables are sometimes fried together, but the beef is in small pieces and is generally well cooked. In restaurants or wealthy homes, a form of native chafing dish is often employed, but in the north, at least, is used only for the cooking of thin slices of fish or mutton. The meat is plunged into boiling water for a minute or two and is apparently completely cooked.

The restaurants and public eating houses are very popular among the Chinese and are frequented especially by those of some means, par-

ticularly when they have only a temporary residence in Peking. In such places the amount of food cooked is very great and the temptation to rush the orders might easily lead to insufficient cooking, especially of meats. Nearly all of the cases of tapeworm infection cited above belong to the classes that frequent such places of amusement and refreshment. The number of students who go there is particularly large. There is surprisingly little data available in the literature that has direct bearing on the problem in hand. Leuckart (1886) summarized the work which had been done prior to 1886 and cited several investigators. Five months after some feeding experiments the cysts were found viable in the muscle of calves without evidence of atrophy or death of any individual. In view of this fact, one could hardly expect that the temporary fattening of cattle in Peking imported from Mongolia would have any appreciable effect in lessening the intensity of the infection. Perroncito found that the cysts do not outlive the host more than 14 days, especially if some degree of putrefaction had taken place. This was observed particularly in the tongue. Soaking in water for 24 hours usually killed the cysts, although the size of the pieces used for experimentation was not stated. Brine was more certainly lethal in its action. Apparently then, "corned beef" should be free from living parasites. Under the heading of Geographical Distribution, Faust (1923) says, "Reported from time to time from various parts of the world, particularly from individuals fond of beef only partially cooked. Occasional reports from various parts of China, but confirmed as indigenous only in North China, where the infection is brought down from Mongolia and Siberia. In and around Peking, more common in foreigners than in natives." A few facts bearing upon the distribution of the worm in the Far East are also mentioned by Leuckart. Baelz wrote him from Japan, saying that *Taenia saginata* was even more common there than *T. solium,* the pork tapeworm. Fedschenko (cited by Leuckart) found the infection widespread in Central Asia and the French soldiers brought it back with them from the Palikao Expedition. Mr. Ma Kiam has kindly furnished some interesting information in regard to this military campaign. In all, the troops were in China about three months, arriving in Tientsin in August, 1860; and they marched toward Peking along the grand Canal. About three weeks later they took Chang Chia Wan, three miles from Tungchow. This town is largely inhabited by Mohammedans who live chiefly on beef and mutton. The battle of Palikao was fought three weeks later and the troops then entered Peking. Being unprepared for cold weather, they concluded an early peace and departed about November 1st. Presumably the army depended largely upon supplies obtained locally. Beef being available in such a community was doubt-

less prepared according to their own methods, which at home lay them liable to infestation. Such infection, therefore, as they contracted evidently had its origin in the vicinity of Peking.

SUMMARY

1. *Cysticercus bovis* from beef offered for sale in Peking, is here reported as the first instance of its observation in China.

2. *Taenia saginata* has been discovered or suspected in 12 cases during 2 years in about 4,500 hospital cases. Two were foreigners and 10 were orientals. All contracted their infection in Peking.

3. The incidence of infection among the native population is probably higher than the records of the servant's and employee's clinic would indicate. Native treatment and indifference to the infestation tend to decrease the number of cases seen.

4. The Chinese methods of preparing beef are apparently effective in most instances, but suspicion points to restaurants in which haste and carelessness in the preparation of food may allow an occasional infection to take place.

5. Careful inspection of thinly sliced meat or the coagulation of muscle protein by heat or the treatment of beef by various methods of preservation are the only safe prophylactic measures.

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A NEW TYPE OF AMOEBA PARASITIC IN MAN OBSERVED IN NORTH CHINA*

ERNEST CARROLL FAUST

So much has been written of late years on human entamoebae, while so little of this great body of data has been found authentic, that one weighs his information for some time before consenting to place it on record. Particularly is this true with regard to creating new species of amoebae pathogenic in man. Dobell (1919) has done an admirable piece of work in showing that the only proved human pathogenic amoeba is *Entamoeba dysenteriae* (or, as he prefers to term it, *E. histolytica*). Yet the form that has come under my observation on four different occasions[1] this year has proved of such decided interest and uniqueness that I desire to communicate my information to others, believing that they, too, may have occasion to observe it.

CASE HISTORIES

CASE 1.—A Chinese farmer of Chihli Province, aged 44, was admitted to the Peking Union Medical College Hospital on September 19, 1922, with an acute case of dysentery. He claimed to have had a dysentery for ten days, but gave no previous history of any such infection. On microscopic examination of the stool large numbers of red and white blood cells were observed and among them many entamoebae, not *Entamoeba dysenteriae*, with red-blood-cell inclusions. A diagnosis of amoebic dysentery was made and the patient placed on emetine treatment, in the form of emetine bismuth iodide, 0.2 gram per diem for twelve consecutive days. Beginning with the fourth day following the first administration of the emetine the stools were negative for amoebae or amoebic cysts and continued negative through a daily routine examination of all stools passed for the next ten days, at the end of which time the patient was dismissed as cured.

CASE 2.—A European, thirty-eight years of age, third-class patient, for a number of years resident in China and in straightened circumstances, was admitted to the Peking Union Medical College Hospital on December 14, 1922, with an acute case of dysentery. He had first noticed the symptoms three months previous, during which time there had been intermittent blood and mucus in the stools and pain at time of defecation. Only on the fourth day following admission did microscopic examination of the stool reveal large numbers of entamoebae, not *Entamoeba dysenteriae*, with red-blood-cell inclusions. A diagnosis of amoebic dysentery was made and the patient was placed on emetine treatment. Emetine bismuth iodide pills (0.2 gram) were first prescribed but were passed undigested in the stool, following which 0.06 gram of emetine hydrochloride was administered intravenously. On the eighth day following admission the microscopic examinations were negative and continued so until the end of the examination some ten days later.

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* Contribution from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College. Read in the Parasitology Section, C. M. M. A., Shanghai, February 16, 1923.

1. A colleague in private practice in Peking has recently observed this same parasite from examination of one of his cases.

Case 3.—A Chinese, aged 28, occupation unknown, of Chihli Province, was admitted to the Peking Union Medical College Hospital on October 2, 1922, with a chronic dysentery. He gave a history of dysentery five years previous to admittance, and had suffered intermittently from digestive troubles since that time.

The stool when submitted to the clinical laboratory proved positive for dysentery bacilli of the Flexner type, and on the fifth day following admission revealed on microscopic examination, specimens of *Entamoeba dysenteriae, E. coli,* numerous flagellates, and also the new entamoebae to be described later in this paper. The patient was placed on a treatment of emetine bismuth iodide (0.2 gram per diem) for a course of twelve routine treatments. On the tenth day after admission the stools were negative for protozoa, while numerous examinations for two months following failed to show any of the new type of amoeba although the stools were positive for *E. dysenteriae* cysts.

Case 4.—A Chinese policeman, aged 42, from Peking, West City, was admitted to the Peking Union Medical College Hospital on October 4, 1922, suffering with acute dysentery. Three years previous to admission he admitted having had dysentery, although his present symptoms had first been noticed three months ago.

Microscopic examination of the stools revealed swarms of *Entamoeba dysenteriae,* and among them small numbers of the new species. The patient was placed on a treatment of emetine bismuth iodide (0.2 gram per diem) for twelve treatments. On the fifth day following admission the stools were negative for the new form and on the sixth and succeeding days they revealed no *E. dysenteriae.* However, the patient died on the eighth day of cardiac failure.

DESCRIPTION OF THE AMOEBA

The amoeba common to all these examinations in the inactive state (Fig. 1) was at first confused with *Entamoeba coli,* although it was measurably smaller than that species. When not quiescent, it measured from 16 to 17μ in diameter, but was longer and more attenuate in the motile state. In Cases 2 and 4, where *E. coli* was also present, an opportunity for comparison with it was afforded, while in Cases 3 and 4, where *E. dysenteriae* was the major amoebic infection, comparison between the new form and the common dysentery amoeba was made possible.

The ectoplasm of the new form was clear and limpid and seemed to be confined to occasional pseudopodia. The endoplasm was thick and viscous. Like *Entamoeba dysenteriae* the Chinese species contained red blood cells (Figs. 21, 22). In Cases 1 and 2 it was the only known responsible agent for the dysenteric condition. Like *E. coli,* but unlike all authentic descriptions of *E. dysenteriae,* it contained at times bacteria within food vacuoles. Unlike both of these or any other described entozoic amoeba it had a definite, fixed polarity, so that there was a definite anterior end, broadly lobose in characteristically active specimens (Fig. 2); it had also a definite posterior end, away from which the organism always tended to flow. Careful examination of this posterior end showed it to be attenuate, with one definite median posterior protoplasmic caudostyle, surrounding which were apparently found at

times several smaller protoplasmic projections. The caudostyle was observed without difficulty in living specimens (Fig. 3) and could be identified in stained specimens fixed in Schaudinn's fluid (Figs. 4, 23, 24). Just anterior to it the cytoplasm was at times in fixed specimens highly vacuolate. A characteristic of this species was the entanglement of the posterior end in a mass of fecal débris, so that individuals in active motion frequently presented the effect of a viscous object dragging a mass of straw along behind it. The caudostyle and the accompanying protoplasmic projections were undoubtedly responsible for this débris entanglement. They not only increased the load of the organism, but contributed, no doubt, to the elongate shape of the amoeba during rapid movement.

In order to study the movement of the organism with respect to its anteroposterior polarity, a specimen freed of débris and without red corpuscle inclusions was placed on a warm stage and observed for a period of 160 seconds, during which time free-hand sketches of its movement and shape were made (Figs. 5-20). An examination of the sketches demonstrates five points: (1) The movement is always away from the caudostyle; (2) the caudostyle is definitely attenuated (i. e., a definite anatomical structure) even when it is not enmeshed in débris and the body consequently elongated; (3) the anterior end is characteristically broadly lobose; (4) the nucleus always lies in the anterior portion of the organism; and (5) pseudopodia, which consist solely of ectoplasm, occur but seldom. In case the tactic stimulus of the animalcule proceeds from the posterior end, as apparently is the case at times, the amoeba then turns on the caudostyle as a pivot, either twisting around it, as in Figure 3, or turning over it in a plane which is vertical to the observer.

The nucleus is a spherical body in the anterior part of the amoeba. It measures 3 to 4.5μ in section, and is provided with minute stipplings of chromatin on the inner side of the nuclear membrane, which, in stained fixed specimens, are seen to be connected with achromatic fibrils. The karyosome likewise differs materially from that of *Entamoeba dysenteriae* or *E. coli*. It consists of a star-shaped clump of chomatin, wih a hollow center, in the center of the nucleus, with radiating lines of chromatic granules. The nuclear structures are, therefore, specifically different from those of either *E. coli* or *E. dysenteriae*.

In vitro the organism does not survive 40 C., even precystic individuals succumbing at this temperature. Since the temperature of the rectum at times exceeds this amount in amoebic dysentery, it seems probable that under such conditions this amoeba must succumb. While it seems altogether probable that the organism follows the course of

all related forms in possessing a cystic stage, in many examinations made, incident to the study of this species, no cysts were found. Division of the organism has not been observed.

Preservation of cover-glass films with Schaudinn's fluid is extremely difficult. Specimens from three of these cases were in most instances poorly preserved, although *E. dysenteriae* and *E. coli* on the same slides gave excellent demonstrations.

DISCUSSION

I have not been able to discover in the literature any reference to an amoeba, either parasitic or free-living, with the essential characters of the species under consideration. While the free-living species, *Amoeba limax* auct., is usually figured with one end considerably broadened and the other narrowed and covered with villous ectoplasmic structures, authorities on the group, like Doflein (1916:676), make no further commitment than that this species, in moving forward, assumes a finger-like contour. No statement is found to the effect that the organism always preserves this same polarity. On the other hand, no parasitic amoeba is described other than the type designated as *Councilmania lafleuri* (Kofoid and Sweezy, 1921), which has been observed to be at the same time phagocytic for red blood corpuscles and bacteria. Even without any specific knowledge of the life cycle of the Chinese species, there is no likelihood of confusing these two forms.

While certain specimens of dysentery amoebae from Cochin, China, figured by Noc (1909, Pl. 10, Figs. 15, 20, 21, 22, 23) might, from their contour, be regarded as belonging to the species herein described, the position of the nucleus with reference to the anteroposterior axis is essentially different.

Minchin (1922:219) raises the question as to the possibility of such forms as *Amoeba limax* being only physiological varieties of other (and, perhaps, less differentiated) species. While that same note of caution may well be kept in mind in the present discussion, there is no evidence that this form in the amoeboid stage with such a pronounced polarity should revert to a more primitive stage, such as Valkampfia does, in which species the differentiation is a flagellation at the anterior end in the retro-amoeboid condition.

Because of the uniqueness of this species of entamoeba, it seems necessary to regard it as a new species of dysentery-producing protozoan, and to create for it a new genus, Caudamoeba, designating the species as *Caudamoeba sinensis*.

CAUDAMOEBA nov. gen.

Entamoeba, measuring 16-17μ in diameter when contracted; parasitic in man; with anteroposterior polarity. Anterior end broadly

lobose, posterior region more narrowed, posterior extremity drawn out into caudostyle, surrounded by villous protoplasmic projections. Posterior region highly vacuolate. Nucleus spherical, measuring 3 to 4.5μ in diameter; nuclear chromatin consists of minute particles distributed over inner surface of nuclear membrane, and connected by delicate achromatic fibrils; karyosome delicate, star-shaped clump with hollow center, lying in center of nucleus which is always near the anterior margin of organism. The amoeba is capable of ingesting both red blood cells and bacteria and is the etiological agent of a dysentery.

Type and only known species, *Caudamoeba sinensis,* with the characteristics of the genus.

Distribution: Reported thus far only from North China.

CLINICAL ASPECTS OF THE SPECIES

There seems little likelihood of escaping the conclusion that the organism, *Caudamoeba sinensis,* is an etiological entity responsible for amoebic dysentery. In all four cases under consideration, the organism contained red blood cells. In the first two cases it was the only suspicious organism found over a period of ten days during which time samplings of every stool passed were examined. In both Case 1 and Case 2 the dysentery abated with the disappearance of this organism from the stool. In Case 3 and Case 4 the preponderant infection with other pathogenic organisms is sufficient to explain the condition following the disappearance of *C. sinensis* from the stool. *Caudamoeba sinensis* is apparently killed by emetine treatment. In Case 1 the organisms disappeared from the stools and the dysentery abated four days after the first treatment with emetine bismuth iodide, and did not appear on subsequent examinations. In Case 2 it disappeared on the sixth day following the first diagnosis and treatment, although the condition was greatly improved on the fourth day. In Case 3 and Case 4, the new species disappeared several days before *E. dysenteriae* was negative in the stool, while in Case 1, Case 2, and Case 3, where follow-up examinations were made no recrudescense of *C. sinensis* infection was found. The drug administered is, therefore, not only a specific for the infection, but is apparently even more successful than it is in *Entamoeba dysenteriae* infection.

The seat of the infection is not known except from clinical symptoms, which all point to its residence in the tissues of the large intestine.

SUMMARY

1. *Caudamoeba sinensis,* nov. gen. nov. spec., a new amoeba from man, is described.

2. The organism is characterized by viscous endoplasm, thin layer of ectoplasm, antero-posterior polarity, lobose anterior and attenuate

posterior end drawn out into a caudostyle, which is surrounded at times by a group of protoplasmic projections more or less conspicuous, a constant movement of the organism away from the caudal end, and a nucleus near the anterior end of the body with minute chromatin granules just within the nuclear membrane, together with a karyosome specifically different from that of the described entamoebae of man.

3. The organism was found in fecal examination of four dysentery patients in the Peking Union Medical College Hospital, all from North China. It is phagocytic for red blood corpuscles and for bacteria and in two instances was the only microörganism in the stool suspected of being pathogenic.

4. Cases containing only this organism in the stools gave typical history and symptoms of amoebic dysentery.

5. Emetine is a specific for the infection. The organism succumbs in the course of a few treatments and does not reappear in the stool after a course of treatments. In this respect it is apparently more amenable to treatment than *Entamoeba dysenteriae*.

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Explanation of Plate XXII

(Figs. 1-4)

Fig. 1.—*Caudamoeba sinensis*, quiescent organism, showing *n*, nucleus, *r*, red-blood-corpuscles, *v*, vacuales, *f*, food inclusions. The caudostyle *c* is surrounded by *d* a mass of débris.

Fig. 2.—Elongate motile specimen.

Fig. 3.—Specimen of *Caudamoeba sinensis* pivoting around the caudostyle.

Fig. 4.—Pre-cystic stage of *C. sinensis*.

Figs. 5-20.—Free-hand sketches of *C. sinensis*, at intervals of ten seconds, showing relation of nucleus and caudostyle to the antero-posterior axis of the organism.

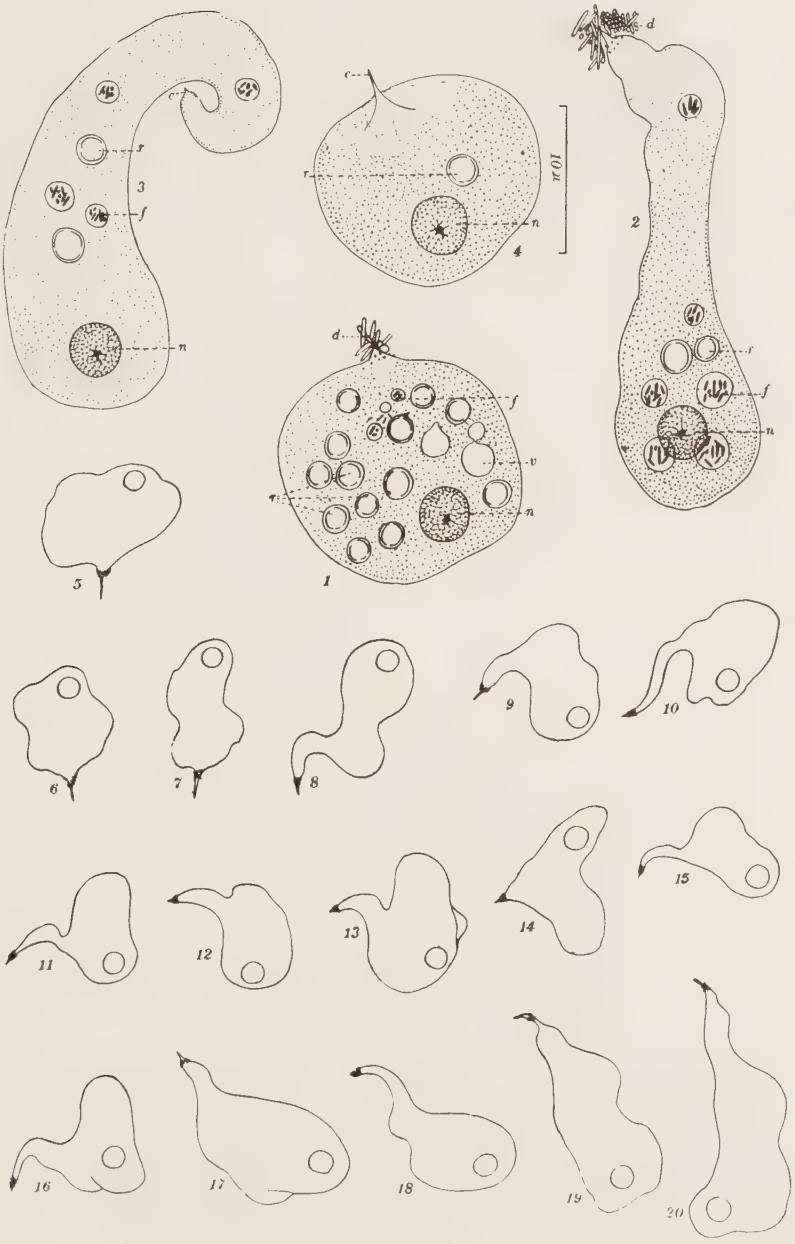


PLATE XXII

EXPLANATION OF PLATE XXIII

Figs. 21 and 22.—Instantaneous photomicrographs of *C. sinensis*, showing red-blood corpuscles.

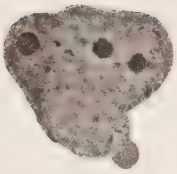
Fig. 23.—Photomicrograph of fixed specimen of *C. sinensis* killed in active condition. Stained with iron-alum hematoxylin.

Fig. 24.—Photomicrograph of fixed specimen of *C. sinensis*, killed in active condition. Stained with iron-alum hematoxylin. Note the character of the karyosome.

Fig. 25.—Photomicrograph of fixed specimen of *C. sinensis* elongated, killed while quiescent. Note nucleus at anterior end.

Fig. 26.—Photomicrograph of fixed specimen of *C. sinensis*, pre-cystic condition. Note nuclear chromatin and central karyosome. The dark mass at the posterior end is an area of vacuoles.

All the photomicrographs are enlarged 1300 diameters.



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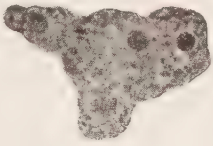

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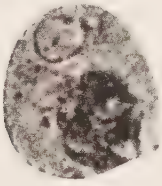

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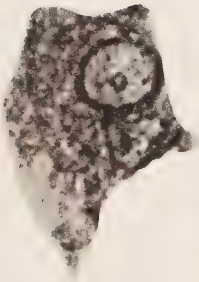

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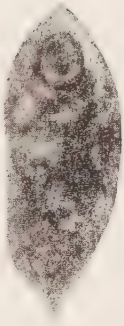

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PLATE XXIII

NOTES ON LARVAL CHARACTERS IN THE GENUS SARCOPHAGA*

Francis Metcalf Root

One finds, from time to time, in the medical literature, reports of fly larvae or maggots as intestinal parasites of man. Probably, in the majority of such cases, no further attempt has been made to determine the exact identity of the larvae. However, in a considerable number of cases where such specimens have been submitted to specialists for determination, it is reported that they were found to be larvae of flesh-flies belonging to the genus Sarcophaga.

Since this genus includes, in North America alone, over 140 species, of widely varying larval habits, it becomes of some importance to determine just what species are concerned in the parasitism referred to. At present it is impossible even to guess at the specific identity unless adults are bred out. As Dr. Aldrich says in his Monograph on *Sarcophaga and Allies in North America*, "almost no specific characters are known in the larvae, so the identification can go no further than '*Sarcophaga* sp.' unless the adult is reared." I hope to show in this paper that this state of affairs is by no means necessary.

For several years I have been spending some of my spare time in collecting and rearing the larvae of our common muscoid flies, in order to obtain material for a study of the characteristic variations in the structure of the spiracles, or external openings of the tracheal system, in these larvae. In the course of this work I have incidentally obtained specimens of the larvae of nine species or varieties of Sarcophaga, of which seven have been reared to the adult stage and identified. These are all common species, whose larvae live either in fecal matter or in decaying meat. An examination of even this scanty material shows that there are some decided differences between the spiracles of different species or groups of species.

The posterior spiracles of muscoid larvae are a pair of dark-colored plates, conspicuously situated on the truncate posterior end of the body. In third-stage (nearly mature) larvae each of these plates consists of a chitinous ring, completely or nearly completely encircling the plate, and inside this ring three slits of variable form, which are the actual apertures through which the tracheal system communicates with the exterior. Each of these slits is divided up into a number of small apertures by a series of chitinous bars.

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In the genus Sarcophaga, these spiracles are placed at the bottom of a shallow pit on the posterior end of the larva. Their shape is also characteristic for the genus. The ring is incomplete, the break coming in the lower inside corners, and the slits, which are almost straight, are much more nearly vertical in position than in any of the other genera.

All this has been known for a long time. However, I have found in my studies that there are in the genus Sarcophaga at least two very distinct types of posterior spiracles, although both types agree in the characters given above for the genus. In the more common type (see Plate I, *S. communis*) the inner portion of the ring is nearly vertical and ends freely in a prominent knob, while the slits are slender and are crossed by a large number of anastomosing bars. In the other type

TABULATION OF THE CHARACTERISTICS OF THE SPIRACLES OF SOME LARVAE OF THE GENUS SARCOPHAGA

	Number of Branches of Anterior Spiracles	
	Range from	Average
Posterior spiracles of *communis* type		
Sarcophaga securifera Villeneuve	10 to 13	11.4
Sarcophaga assidua Walker	12 to 16	13.9
Sarcophaga sarracenioides Aldrich	14 to 16	15.0
Sarcophaga communis Parker	16 to 20	18.2
Sarcophaga bullata Parker	17 to 21	18.3
Sarcophaga haemorrhoidalis Fallen	17 to 19	18.0
Posterior spiracles of *ochracea* type		
Sarcophaga communis var. *ochracea* Ald.	18 to 23	20.5
Sarcophaga "G"	17 to 19	17.8
Sarcophaga "F"	30 to 35	32.6

(see Plate I, *S. Communis* var. *ochracea*) the inner portion of the ring curves outward and tapers to a point, ending in contact with the lower end of the inner one of the three slits, and the slits themselves are broader than in the first type and are crossed by a much smaller number of distinct bars. To illustrate this difference I have chosen *S. communis* Parker and its variety *ochracea* Aldrich, in order to emphasize the fact that two forms which are almost indistinguishable as adults may be very distinct as larvae.

Specific differences are also found in the anterior spiracles. These are located on the sides of the second (apparent) segment of the larva, and each consists of a short, flattened tube which splits up externally into a variable number of short lobes or branches, each of which seems to have a small aperture at its tip. The number of these branches seems to vary within certain definite limits for each species (see Figure 1), as is indicated in the table.

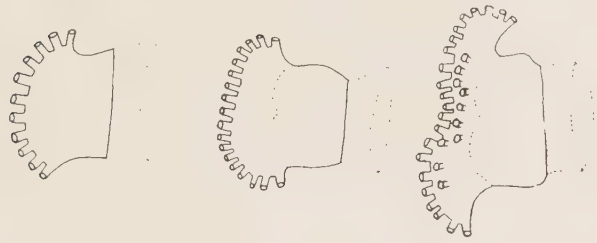

Fig. 1.—Anterior larval spiracles of *Sarcophaga securifera* Villeneuve (left), *Sarcophaga bullata* Parker (middle), Sarcophaga "F" (right)

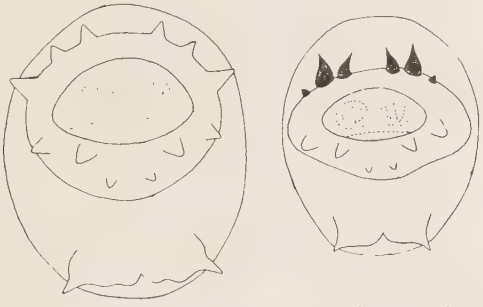

Fig. 2.—Diagram of posterior end of larvae of *Sarcophaga bullata* Parker (left), Sarcophaga "G" (right).

Photographs of the posterior larval spiracles of *Sarcophaga communis* Parker (left), *S. communis*, var. *ochracea* Aldrich (right).

PLATE XXIV

In one of my unidentified species (recorded in my notes as Sarcophaga "G") there is an even more prominent point of difference. In all Sarcophaga larvae that I have seen, the pit which shelters the posterior spiracles is surrounded by a raised rim which bears a definite number of processes or tubercles. In most larvae the three pairs of tubercles on the dorsal portion of this rim are white and fleshy. In the larvae of Sarcophaga "G" these three pairs of tubercles are close-set, heavily chitinized and almost black in color (see Figure 2).

I have collected larvae of this type twice, at Woods Hole, Mass., and at Salisbury, Md., on both occasions in isolated deposits of human excrement. There are slight differences between the two sets of larvae (the Woods Hole form is the one figured) and they may belong to two distinct, but closely related, species. Possibly they are the larvae of *S. latisetosa* Parker and *S. quadrisetosa* Coquillet, but this is only a guess, since I have not succeeded as yet in rearing the adult.

In most of the Sarcophaga larvae which I have examined, the cuticula, or outer covering of the body, is comparatively smooth, except for the conspicuous spinose areas on each segment. But in the larva of *S. assidua,* the whole posterior two-thirds of the body is thickly covered with slender, hair-like, chitinous projections, which are particularly pronounced on the tubercles around the depression in which the posterior spiracles lie. This may prove to be a valuable character for distinguishing the larvae of this species from those of the others which breed in human excrement.

The combination of these various characters permits of a satisfactory identification of the larvae of several species of Sarcophaga, so far as the material at my disposal is concerned. It must be remembered, however, that my material is by no means complete, even for the dung and carrion inhabiting species found in the vicinity of Baltimore.

COMPLEMENT-FIXATION TEST OF *SCHISTOSOMATIUM PATHLOCOPTICUM* AND ITS GROUP REACTION WITH *SCHISTOSOMA JAPONICUM*

BUNSHIRO TANABE

From the Department of Tropical Medicine, Harvard Medical School, Boston, Mass.

In 1919 Fairley devised an ingenious complement-fixation test for Bilharziasis, by employing as antigen an extract of infected snails' livers. The antigen was prepared by macerating a number of livers containing cercariae of *Schistosoma mansoni* either in absolute alcohol or in a solution composed of 0.85 per cent. saline and 0.5 per cent. phenol. Recently Le Bas (1922) studied the nature of the antigen in the complement-fixation test for Bilharziasis. By testing the reaction with antigens prepared in various ways he concluded that the antigenic substance, acting in the test for Bilharziasis, is insoluble in absolute alcohol and acetone, and soluble in normal saline solution and also in 50 per cent. alcohol diluted with physiological saline. By employing as antigen an extract of adult worms of *Schistosoma japonicum* Tanaka (1912) obtained positive results in a complement-deviation test in a rat with infection of over one month's standing.

In November, 1922, I prepared an antigen by macerating five livers of the snails, *Lymnaea palustris,* infected with the cercaria of *Schistosomatium pathlocopticum* in 5 cc of 50 per cent. alcohol diluted with 0.85 per cent. saline solution. This mixture was well shaken and incubated at 37 C. for 24 hours; it was then filtered and the filtrate used as antigen. By employing this antigen the complement-fixation reaction was carried out with the serum of a white rat infected with the cercaria of *Schistosomatium pathlocopticum.* On June 5, and 15, 1922, four young white rats were exposed to the cercariae and successfully infected. In the course of two months three of the four rats died of acute schistosomiasis, since many adult worms and ova of *Schistosomatium pathlocopticum* were found in the liver and the wall of the intestines.

Only one of the four rats survived. This rat became very emaciated, the hair came off on the posterior part of the body, although the rat showed no loss of appetite. On November 13, about 2 cc. of blood was drawn from the heart of this rat. The serum was inactivated by heating at 56 C. for 30 minutes. The anticomplementary dose of the antigen was titrated. In the following main tests the antigen was diluted so that 0.1 cc. of the dilution contained less than one half the maximum amount which did not inhibit hemolysis. Complement was

freshly obtained from two healthy guinea pigs, and its minimum hemolytic doses (MHD) was determined by a method of titration. In the main test, three, five and seven MHDs were used. In Fairley's work the total volume in the final stage of the reaction was 0.5 cc. (five volumes), and each unit volume equaled 0.1 cc. The arrangements and the results of the tests are shown in Table 1.

According to Fairley's description these results are to be recorded as "P + +." This means "definitely positive." Thus positive comple-

TABLE 1.—*Complement-Fixation Test in a White Rat Infected with the Cercaria of Schistosomatium pathlocopticum*
(Performed on Nov. 14, 1922)

	Tube	Antigen 50% alcohol extract of infected snail liver (1:16) cc.	Antibody white rat serum inactivated cc.	Complement (0.1 cc.) M H D	Sufficient normal saline solution to bring the total volume to 0.3 cc.; tubes were gently shaken and incubated at 37° C for one hour	Hemolytic mixture 5% suspension of sheep blood corpuscles plus amboceptor (2 units) cc.	Tubes shaken and incubated two hours, preliminary reading	Results: Final readings were made after being kept in ice box over night
Antigen control	1	0.2	0	3		0.2		Hemolysis
Serum control	2	0	0.1	3		0.2		Hemolysis
Serum test...	3	0.1	0.1	3		0.2		Complete inhibition of hemolysis
Serum test...	4	0.1	0.1	5		0.2		Complete inhibition of hemolysis
Serum test...	5	0.1	0.1	7		0.2		Marked inhibition of hemolysis
			Normal rat's serum inactivated cc.					
Normal serum control	6	0.1	0.1	3		0.2		Hemolysis
Normal serum control	7	0	0.1	3		0.2		Hemolysis
Complement control	8	0	0	1		0.2		Hemolysis
Complement control	9	0	0	3		0.2		Hemolysis
Complement control	10	0	0	5		0.2		Prompt hemolysis
Complement control	11	0	0	7		0.2		Prompt hemolysis
Hemolytic system control	12	0	0	0		0.2		No hemolysis

ment-fixation reaction was obtained in one case in a rat which stood about five months' infection with *Schistosomatium pathlocopticum*. The white rat from which the blood had been drawn for the antigen test died on the next day. The autopsy was made; great numbers of degenerated and deformed ova were found in the liver and the wall of the intestine. This rat had surely been suffering from chronic schistosomiasis.

The antigen test introduced by Fairley serves two important purposes: (1) as an aid in diagnosis, particularly in those cases of

schistosome infection where the eggs of the parent worms are few or difficult to find, and (2) as a guide in the treatment of schistosomiasis just as does the Wassermann reaction in syphilis. It is of great interest to know that such an antigen prepared from *Physopsis africana* infected with the cercariae of *Schistosoma bovis* has been shown by Dr. W. A. Murray (1921) to give positive reaction in sheep harbouring either *S. haematobium* or *S. japonicum*.

In November, 1922, Drs. K. Miyairi and K. Morishima kindly sent me great numbers of the Japanese snails, *Blanfordia nosophora*, infected with the cercariae of *S. japonicum*. The vast majority of the snails were still alive over one month after the collecting and send-

TABLE 2.—*Complement-Fixation tests for White Rats No. 2 and No. 3 Infected with Cercaria of S. japonicum*
(Performed Dec. 28, 1922)

	Antigen (1:16) cc.	Anti-body inacti-vated cc.	Comple-ment (0.1 cc.) M H D	Normal saline solution q. s. 0.3 cc. in the total volumes; tubes shaken and incubated at 37 C for one hour	Hemolytic mix-ture 5% suspen-sion of sheep blood corpuscles plus amboceptor (2 units) cc.	Tubes shaken and incubated at 37 C for two hours	Results: Final readings were made after being kept in ice box over night
Antigen. control..	0.2	0	3		0.2		Hemolysis
Rat No. 2	0	0.1	3		0.2		Hemolysis
	0.1	0.1	3		0.2		Slight inhibition of hemolysis
	0.1	0.1	5		0.2		Slight inhibition of hemolysis
	0.1	0.1	7		0.2		Almost complete hemolysis
Rat No. 3	0	0.1	3		0.2		Hemolysis
	0.1	0.1	3		0.2		Marked inhibi-tion of hemol.
	0.1	0.1	5		0.2		Slight inhibition of hemolysis
	0.1	0.1	7		0.2		Slight inhibition of hemolysis
The other seven controls were conducted in the same manner as in Table 1							The same right results as in Table 1

ing. The cercariae were found in about 3 per cent. of the living snails. Four young white rats were infected with the cercariae escaping from the snails. By utilizing the same antigen as used in the previous test I carried out complement-fixation reaction for two of the four white rats five weeks after infection. The system and arrangements of the tests were the same as in the previous test. As seen in the accompanying table (Table 2), the results were weakly positive. But it is noteworthy in this connection that in these two cases the tests were made five weeks after infection with cercariae of *S. japonicum*, whereas in the former case the test was made about five months after infection with *Schistosomatium pathlocopticum*. In the former case the freshly prepared antigen was used. In the latter cases the same antigen was employed forty-five days after its preparation.

SUMMARY

By employing as antigen an alcohol extract of *Lymnaea palustris* infected with the cercaria of *Schistosomatium pathlocopticum* complement-fixation tests introduced by Fairley were made for one white rat which stood about five months' infection with the same parasite and also for two white rats which stood five weeks' infection with *S. japonicum.*

The results of the former test were definitely positive, and those of the latter tests were weakly positive, whereas normal rat serum controls both with and without the antigen always gave negative results.

In the complement-fixation reaction there are certain immunological relations between *S. japonicum* and *Schistosomatium pathlocopticum,* and the same relationship has been confirmed among *S. hacmatobium, S. mansoni, S. japonicum* as well as *S. bovis.* Therefore, infection with any species of these schistosomes produces an antibody in the blood of the host, which yields more or less positive results in the complement-fixation test for the schistosomiasis even of other species than the schistosome producing the infection, i. e., a group reaction.

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THE OCCURRENCE OF *ANCYLOSTOMA BRAZILIENSE*, DE FARIA 1910 IN THE PHILIPPINE ISLANDS

SAMUEL T. DARLING

Ancylostoma braziliense more commonly known as *A. ceylanicum* was first described by Gomez de Faria in 1910, from cats and dogs near the City of Rio de Janeiro, Brazil. The following year Looss (1911) described what is undoubtedly the same species under the name *A. ceylanicum*. Lane (1913) recorded finding the species in man in Bengal, India. The writer from the examination of material from Indonesia, Fiji, West Africa, Panama and Brazil is of the opinion that while the size and morphology of the worms vary slightly in different regions, and from different hosts, as well as in the individual host, there appears to be no specific difference between the forms described as *A. ceylanicum* and those described as *A. braziliense*, an opinion already reached by Gordon (1922).

The Uncinariasis Commission to the Orient made a study of the effects of hookworm infection on the working efficiency of the people of the Malay Peninsula, Java, and Fiji. In this study large numbers of worm counts were made of hookworms expelled from natives of these countries. Very careful records were made of the species and sex of the hookworms expelled from the persons treated and examined. A few specimens of *A. braziliense* were found in natives of the Malay Peninsula, Sumatra, Java, China, and Fiji. These were undoubtedly derived from dogs because the dogs of the locality were found to be more or less severely infected with this species. *A. braziliense* has also been reported from West Africa, South Africa, Siam, and Amazonas, Brazil. It is not so specific in its host relationships as the more common species of hookworms, for it is found in man, dog, cat, lion and civet cat. Wherever *A. braziliense* occurs in large numbers in the dog or cat, there one may expect to find a few cases of human infection. Hitherto this species has not been reported from the Philippines although Wharton (1917) examined 118 dogs from the City Pound in Manila and found as many as 300 or 400 hookworms in some dogs, the only species found being *A. caninum*.

Through the kindness of Dr. C. V. Leach I have had an opportunity of examining a number of hookworms from a pointer pup from Manila, P. I. The worms are of two species, *A. caninum* and *A. braziliense*. The total number of worms being about 480 of which approximately 37 per cent. are *A. braziliense* and 63 per cent. are *A. caninum*. After a

preliminary comparison had been made with microscope, *A. braziliense* could easily be distinguished from *A. caninum* with the naked eye, by the smaller size and delicacy of the former.

The worms had been in spirit for more than a year and all had the same general color. None of the specimens of *A. braziliense* presented the hyaline anterior fifth so characteristic of specimens in the fresh state. The mouth parts of some specimens of *A. braziliense* were found to possess a peculiar character seen in some specimens from Sierra Leone, Brazil and Panama in that one of the large pair of teeth sometimes both members of the pair had truncated ends, thus giving the tooth a plate-like rather than a tooth-like appearance. This peculiar feature of *A. braziliense* is very common in specimens from Brazil but so far as is known no attention has been called to it. The smaller pair of ventral teeth are always of good size, and in this respect resemble the type seen in the Malay Peninsula. The dorsal rays presented the usual characters of this species and could be differentiated easily from those of *A. caninum*.

It may be observed that specimens of *A. braziliense* recovered from man have been found with bright red blood in the intestinal tract a circumstance noted also in specimens of *A. duodenale* but never in *Necator americanus*. From the fact that *A. braziliense* has been found in rather large numbers in a dog from the Philippines, cases of human infection by this hookworm will most certainly be encountered in the same region. It is unlikely however that any cases of severe infection will occur for man rarely harbors more than three or four worms of this species.

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SOCIETY PROCEEDINGS

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The sixty-second meeting was held October 21, 1922. Mr. J. R. Christie was elected an active member.

Dr. Cort spoke briefly on hookworm investigations carried out in Porto Rico during the summer by himself, Dr. Riley, Dr. Payne, Mrs. Payne, Mr. Stoll and Mr. Augustine, the results of which will appear in a series of papers on hook-worms in the *American Journal of Hygiene.*

Mr. Stoll described a method for the determination of the number of hookworm eggs in a given sample of feces. After balancing the sample on the scales 3 gms. are removed to a centrifuge tube graduated to 45 c.c. which is then filled to 45 c.c. with N/10 NaOH, plugged with a rubber stopper and thoroughly shaken. While the material is in suspension, 0.15 c.c. is taken in a pipette and transferred to a slide. The number of eggs on the slide multiplied by 100 gives the egg count per gram of the original sample. Two counts are made and if the discrepancy between counts is too great, the operation is repeated. The sodium hydroxide solution renders the eggs less sticky, and has other favorable effects, including deodorization of the feces and at least partial saponification of fecal fats.

Dr. Cobb gave the following notes:

Observations on Nemas

Salivary Glands of the Nemic Genera Tylenchus and Aphelenchus.—Tylenchus and Aphelenchus, two important nema genera containing species of great economic significance on account of their destructiveness to their hosts, resemble each other in many ways—anatomical, physiological, and ecological. In fact, in some cases, it has not always been possible to distinguish with precision between these two genera, especially in the young state, and not infrequently observers have referred specimens to the wrong genus. Precise knowledge of the differences is more important than it would be in genera of less economic significance.

A study of the salivary glands of a number of species appears to make evident in their structure an additional contrast between these two genera. The salivary glands of Tylenchus have been described and figured by the writer—especially those of *T. similis* and *T. penetrans.* Examination of the corresponding organs in *T. tritici* and *T. dipsaci* discloses the same type of structure, namely, the presence of three, more or less amalgamated unicellular salivary glands lying in a "tandem" series in the base of the neck and emptying forward through separate ducts, one of them at the base of the spear, the other two at the base of the valve of the median esophageal bulb. Similar glands are frequently met with in Aphelenchus and are a normal feature in that genus. They occur, for instance, in *A. modestus* deMan, *A. agricola* deMan, and in five other as yet unpublished species known to the writer, but the ducts leading forward from the glands empty in a different way, one of them, denominated dorsal, passing through the dorsal sector of the median bulb and emptying through a pore located, not at the base of the spear as in Tylenchus, but in the front part of the median bulb; the other two emptying as in Tylenchus, that is, at the base of the valve of this same bulb. In both genera the mouth of the dorsal duct quite frequently is accentuated by a refractive peculiarity of the lining of the esophagus, which latter, it is well known, is usually a rather conspicuous feature in these genera. In Tylenchus

there is an oblique refractive element, minute, but once noticed rather easily seen, extending outward and backward dorsally from the tubular lining of the esophagus just behind the base of the spear, by which the opening in the esophageal lining constituting the mouth of the dorsal gland is clearly indicated, even when the gland itself cannot be seen. In Aphelenchus also a break in the esophageal lining, but not of the same character, exists in the anterior half of the median bulb, where the lining seems to be interrupted for an exceedingly short space. The elements of the lining are, however, so refractive that this interruption, minute as it is, is often quite easily visible. This break marks the mouth of the homologous gland described above as emptying farther forward in Tylenchus. In other words the location of the mouth of the dorsal salivary gland may serve as a generic character (Fig. 1).

The Deirids ("Cervical Papillae") of Nemas.—The writer's recent observations lead to the conclusion that the cervical lateral papillae (*deir*, Fig. 1) are a widespread feature among nemas. Apparently these papillae have no other homologues on the individual nema. Evidence of this is obtainable, for instance, from *Aphelenchus agricola*, where the lateral fields are traversed by fine longitudinal striae, in the midst of which any other similar papillae would be readily detected but where they are sought in vain.

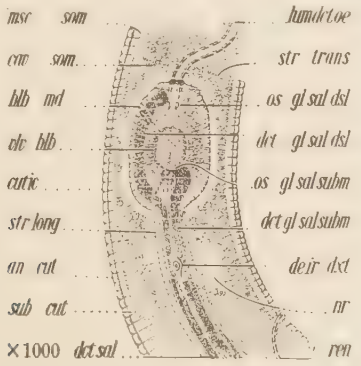

Fig. 1.—Deirid, or cervical papilla, and adjacent structures. *msc som*, somatic muscles; *cav som*, somatic cavity; *blb md*, median esophageal bulb; *vlv blb*, valve of the median bulb; *cutic*, cuticula; *str long*, longitudinal striae on the lateral field of the cuticula; *an cut*, annule of cuticula; *sub cut*, subcuticula; *dct sal*, duct of dorsal salivary gland; *lum dct oe*, lumen of the esophageal duct; *str trans*, transverse stria; *os gl sal dsl*, mouth of the dorsal salivary gland; *dct gl sal dsl*, duct of the dorsal salivary gland; *os gl sal subm*, mouth of the right submedian salivary gland; *dct gl sal subm*, duct of the right submedian salivary gland; *deir dxt*, right deirid; *nr*, nerve ring; *ren*, duct of the renette.

It is quite possible that this pair of cervical papillae of the Tylenchidae, etc., are the homologues of similar structures known, not only on species of other free-living groups, but also on many nema parasites of the higher animals, and that therefore they are of deeper phylogenetic significance in the nema phylum than has been realized. They seem to deserve a more distinctive name than cervical papillae, since various sorts of cervical papillae are known on nemas, and the name *deirids* is therefore suggested.

Revival of Desiccated Nemas.—A new tree-inhabiting Tylenchus, to be described elsewhere, has, in a well-developed degree, a property of great economic significance in the genus Tylenchus, and in some other genera injurious to agriculture—namely, that of reviving after long desiccation. When

portions of certain infested oak galls are placed in water in a cool situation, nemas of this species inhabiting them will revive after a few hours, even when the galls have been in a quite dry condition for several years. On one occasion, nemas inhabiting galls that had been filed in an herbarium in a steam heated building in Washington, D. C., were readily revived after two and one-half years.

This property of reviving after desiccation is now known to be quite widespread among free-living and plant-infesting nemas. The author's researches show that it is not confined to one genus, or to a few closely related ones, but occurs in such widely different genera as Cephalobus, Rhabditis, Plectus, Tylenchus, Aphelenchus, Dorylaimus, Chambersiella, and others. This resuscitation is usually not a generic property but is characteristic only of certain species in each genus. It was long ago known that *Tylenchus tritici* can be revived after twenty or more years of desiccation, and also that *Plectus parietinus* will revive after severe desiccation. It is only in recent years, however, that it has become evident that this property is widespread among nemas. The writer has observed it in numerous species under such circumstances as to convince him that the property is probably far more common than is at present known.

The methods of revival have not been very thoroughly investigated; the trials conducted by Dr. Ritzema Bos, and later independently by the writer, have shown that dried up individuals of *Tylenchus dipsaci,* the well-known destructive parasite of the onion, hyacinth, clover, and a considerable number of other useful plants, can be revived by the application of water. Both series of researches show that the revival can be very gradual, and that even so late as six weeks after immersion individuals hitherto apparently dead continue to revive. It is found that if the nemas are exposed on a chemically clean glass surface in an extremely thin film of tap water in a cool moist chamber, they may be more successfully revived than if wholly immersed. The writer's experiments showed that clean red clover seed from diseased crops, even recleaned seed, that is to say the cleanest seed known to commerce, may carry revivable specimens of *Tylenchus dipsaci* even when more than one year old.

This ability to withstand desiccation is, of course, of very great value to some species of Tylenchus in preserving them from extinction. From what is now known concerning this important matter, one is justified in suspecting that the Tylenchus of the Chilean beach-tree (foliage), a new species to be described elsewhere, will prove to be among those displaying this property, although in the experiments tried on the very few specimens available, none revived. Nothing is known about the method of distribution of these two arboreal nemas. While it is possible that they might reach their habitat through their own muscular exertions, it seems more likely that they are aided by carriers of some sort. Of these carriers our present knowledge would indicate the order or probability to be somewhat as follows: insects, birds, climbing animals including snails, wind, water.

The author has from time to time discovered quite a number of very interesting cases of the transference of nemas by insects. These are now sufficiently numerous to justify the surmise that in the present case if carriers are involved they are much more likely to be insects than otherwise.

It is believed there are no well established cases of purely plant-infesting nemas being transferred through the alimentary canal of insects, though there is reason to suspect that they might be so transferred by certain snails. In many of the known cases, the nemas are transferred by adhesion to the mouth parts or feet and legs of the insects.

Miss Cram reported *Gongylonema ingluvicola* Rans. from the undilated portion of the esophagus of the domestic fowl, a species heretofore recorded only from the crop. The bird in this case was obtained in the Washington market and contained two worms of this species, both in the undilated portion of the esophagus, one being within an inch of the pharynx. Attention was

also called to a drawing of a specimen of *Contracaecum osculatum* from a sea-lion which had threaded itself partially through the neural arch of the neural spine of a fish vertebra (Fig. 2). Numerous cases were cited of nematodes becoming entangled with foreign objects within the lumen of the intestine.

Dr. Cobb noted that in the case of free-living nemas, the mycelium of certain fungi occasionally "strangles" the worms and judging from the microscopic appearance of the fungus, takes nourishment from the worm.

Mr. Christie described a modification of the Cobb differentiator, for use in nematode technique, in which a carrier containing the material is attached by a cord to a drum operated by an ordinary alarm clock. The carrier is thus drawn up through the tube of the differentiator or lowered as the case may require, in a certain definite period of time. The differentiator tube is sealed at the bottom instead of having the capillary opening as in the Cobb form.

Dr. Hall gave the following notes:

A Snail from the Crop of a Chicken.—A Rhode Island Red cockerel between 5 and 6 months old was killed by the writer and when dressed for table use was found to have a specimen of snail, *Polygyra albolabris*, as identified by Dr. Bartsch, in the crop, the snail being in a good state of preservation as regards its soft parts and evidently recently swallowed. In connection with the fact that snails act as intermediate hosts of flukes and have been suspected of being the intermediate hosts of *Davainea bothrioplitis* and *D. echinobothrida*, and that slugs are reported as the intermediate hosts of *Davainea proglottina*, the occurrence of this snail in a chicken is naturally of interest. The most striking feature was the size of the snail. It measured in its greatest diameter 2.75 cm., or a little more than an inch. Browne (1922) says that a sponge larger than a hen egg can be passed to the crop of a chicken. The fact that a snail is quite large is therefore no reason for excluding it as a possible intermediate host of chicken parasites. If a cockerel less than six months old can swallow a snail which is over an inch in diameter, a full-grown bird could doubtless swallow a much larger snail or other object.

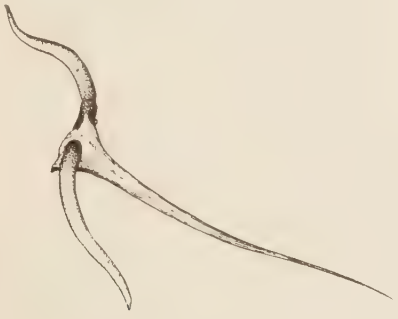

Fig. 2. *Contracaecum osculatum* in arch of neural spine fishbone. ×2. From sea-lion.

Fly Larvae Breeding in a Dead Bot.—In a study of the effects of certain drugs on bots, the examination of some horse manure which had been allowed to stand a day or two disclosed one dead bot, *Gasterophilus intestinalis*, which showed active movement as a result of a number of fly larvae inside the cuticula of the bot. The larvae had entered the bot from the side, very close to the posterior end, and had apparently cleaned out the internal organs of the bot. In this case it was definitely known that the bot had been killed by the drug used (carbon bisulphide) as, in our experience, bots removed by carbon bisulphide are always found to be dead. Lacking this information, one might

have raised the question as to whether fly larvae, *Musca domestica*, could attack and enter a live bot passing spontaneously in the manure on its way to pupation. In this connection it might be noted that some writers give directions for killing bots passed after treatment with carbon bisulphide to prevent pupation. This is unnecessary. Stegman (1920) states that Glaeser has several times found fly larvae in pupae of the ox warble, Hypoderma, the larvae living on the pupal content. Possibly these are cases where the larvae have attacked dead pupae.

The Wandering Habit of Ascaridia perspicillum.—In connection with a recent case of *Ascaridia perspicillum* in a hen egg, I have taken occasion to examine part of the collection of the Bureau of Animal Industry to obtain information in regard to the number of cases there in which these worms were found outside their normal location in the small intestine. The number of cases in which this worm has occurred in unusual situations indicates that it has a wandering habit quite comparable to that of *Ascaris lumbricoides*. It is evident that the worm may wander forward to the gizzard, crop and esophagus, or backward to the large intestine and cloaca, and from the cloaca to the oviduct, where it may become caught in the egg as it is formed, or it may pass through the oviduct to its fimbriated extremity and enter the body cavity.

In the available collection there is evidence that this worm may do all of these things. Specimens collected at Summerville, South Carolina, May, 1913, are labelled as from the esophagus, gizzard, etc. It is evident that specimens passing to the esophagus must go through the crop and I have the impression that I have seen this parasite in the crop or read of such a case. Specimens collected at Riverdale, Md., July, 1910, are labelled as from the large intestine and in view of the records from the hen egg there seems to be no reason for doubting the record. There are specimens from hen eggs collected at Falls Church, Va., May, 1911; Washington, D. C., October, 1911; El Paso, Texas, 1917; an unspecified locality, September, 1919, and from Grand Forks, N. D., September, 1922. There are a number of similar records in the literature but they do not appear to have been summarized. Specimens from Baltimore, Md., and Littlefield, Texas, are labelled as from the abdominal cavity and while all such records are open to suspicion as possibly covering cases where the intestine had been severed in opening the bird, with the subsequent escape of the worms, it seems entirely possible that worms which are known to wander up the oviduct rather frequently might continue the journey, escaping entanglement in an egg in a bird that was not laying and enter the body cavity.

Dr. H. G. May sent an abstract of a paper:

On Killing, Staining and Mounting Nematodes

Parasitologists are mostly following Looss in killing nematodes by means of hot solutions, usually of alcohol. This has the advantage of straightening out the worms as they die. However, the use of concentrated solutions, either hot or cold, so changes the structure of the cuticula as to render it impervious to balsam and balsam solvents. This change can in a large measure be avoided by the use of dilute solutions. A weak solution of ethyl alcohol appears to be the most satisfactory. The string siphon of Magath is preferred by the author to Cobb's differentiator, on the ground that the material is more easily handled. The string is so selected as to permit a change of fluid in from six to twelve hours. The alcohols are increased in strength each time by from five to ten per cent. Staining may be accomplished at any point in the operation of dehydration. Delafield or Boehmer hematoxylin in 70 per cent. is preferred by the author. This is followed by a weak solution of sodium or potassium acetate in 75 or 80 per cent. alcohol. Absolute alcohol is followed by ⅓ xylene in alcohol, ⅔ xylene in alcohol and pure xylene. This is

followed by synthetic oil of wintergreen (methyl salicylate) in which dry Canada balsam is allowed to dissolve until the consistency is right for mounting.

The sixty-third meeting was held at the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md., on Nov. 18, 1922.

Dr. Root read a paper on the larval characters of the genus Sarcophaga (Diptera) which are of use in taxonomy. (To be published elsewhere).

In comment, Dr. Stiles stated that many cases of Sarcophagids parasitic in man have been reported to the Hygienic Laboratory. As most of these cases are from regions where the open privy is in use, it is probable that human infection occurs at the time of defecation, the eggs being laid at or near the anus of the subject.

Dr. Hall gave the following note on the hookworm of swine. Nematodes collected from swine in Samoa and sent in by Dr. Daniel Hunt of the naval medical service were examined in the Zoological Division of the Bureau of Animal Industry and found to be *Crassisoma samoense* Lane, 1922, reported by Lane from swine with the geographical locality given as "Pacific." While hookworms have not been regarded as commonly present in swine or of much importance in these hosts, it appears that at least 8 species of hookworms have been reported from these animals. While the genus Crassisoma Alessandrini is regarded by some authorities as a synonym of Globocephalus Molin, Lane has recently accepted both genera and made a new species in each genus, both of the species in question being from swine. If the species in these genera are accepted as valid and distinct, the hookworms reported from swine are as follows: *Crassisoma urosubulatum, C. samoense, Globocephalus longemucronatus, G. connorfilii, Necator suillus, Ancylostoma duodenale, Uncinaria stenocephala* and *Bunostomum trigonocephalum.* The first five of these species appear to be normally parasitic in swine. Of the last three species, *A. duodenale* is normally parasitic in man, *U. stenocephala* in dogs and foxes, and *B. trigonocephalum* in sheep. The species of *Necator* recently reported from swine in Brazil by Gordon and said to resemble *N. americanus* appears to conform fairly closely to the description of *N. suillus* and may prove to be this species. Dr. Cort discussed the probable occurrence of human hookworms in swine and the close resemblance of *Necator americanus* and *N. suillus*, the latter being common in swine in Trinidad. Dr. Stiles referred to the practical bearing of the occurrence of human hookworms in swine upon hookworm control. Mr. Stoll discussed the relation of the acidity of soil to hookworm infection based on a study of Petri dish cultures of fecal material, each of 3 gms. and containing approximately 9,000 hookworm eggs. The relation of hookworm infection to meat and vegetable diets was discussed by Drs. Cort, Curtice and Stiles.

Dr. Hegner read a paper on the effect of diet on intestinal protozoa. In the rat there are commonly three genera of intestinal protozoa represented, Giardia in the duodenum, Hexamitus in the ileum, and Trichomonas in the cecum. It was found that a carnivorous diet will nearly exterminate Trichomonas in a short time. With a return to the normal or vegetable diet, the normal heavy infestation of the rat with Trichomonas also returns but in a somewhat longer time. It was pointed out that Trichomonas is a feeder on bacteria. The fact that a carnivorous diet will change the bacterial content of the rat's intestine from the ratio of 1 putrefactive and 99 acidophilous bacteria to 99 putrefactive and 1 acidophilous bacteria within 4 to 5 days, is of undoubted significance in relation to the effects of diet on Trichomonas.

Mr. Holmes gave some notes on a species of Balantidium from the South American monkey, *Cebus variegatus.* The species appears to be distinct from those reported from swine and human beings. *Balantidium* sp. has previously been reported from African monkeys. Mr. Holmes also discussed *Entamoeba cobaya* from *Cavia cobaya*, showing drawings of the eight-nucleate cysts. It was pointed out that in the cat *E. coli* often shows cysts but that *E. histolytica* never does.

Mr. Becker read a paper on the specificity of herpetomonad parasites of muscoid flies. He stated that in dealing with strains from seven genera of flies it was possible to transfer any strain to flies of any of the seven genera, indicating that the herpetomonads of different species of flies frequently considered to be of distinct species are in reality of the same species.

Dr. Ransom called attention to the use of the guinea pig as an artificial host for many parasitic nematodes. Guinea pigs have long been used in bacteriological laboratories but their use in the study of metazoan parasites has been limited. Owing to the fact that they are subject to but one intestinal metazoan parasite, *Paraspidodera uncinata,* and that but rarely, the results of experiments are not likely to be confused by the presence of other parasites similar to those under investigation. It has been found that various nematodes, which normally are quite closely restricted in nature in their choice of hosts, may undergo developmental changes and migrate in the guinea-pig in a manner similar to that in their proper host. *Syngamus trachealis,* the gapeworm, when introduced into the guinea-pig in the infective stage will migrate to the lung and develop there into a later stage, certain horse strongyles will establish themselves in the intestinal wall of the guinea-pig and the larvae of the stomach worm of ruminants, *Haemonchus contortus,* when introduced into the guinea-pig will continue their development and grow to a considerable size. Dr. Cort noted that the rat has been used in a similar way in hookworm investigations.

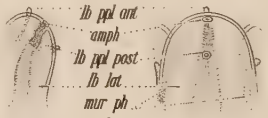

Fig. 3.—Amphids of *Rhabditis cylindrica. lb ppl ant,* anterior labial papilla; *amph,* amphid; *lb ppl* post, posterior labial papilla; *lb lat,* lateral lip; *mur ph,* wall of the pharynx.

Mr. Augustine discussed the death of hookworm larvae in cultures. The greatest reduction in the number of live larvae occurred during the first week and that the death rate was lowest in those cultures in which the longest lived larvae were.

Dr. Cobb presented the following notes:

Interesting Features in the Anatomy of Nemas

Attention is called to the existence of amphids in *Rhabditis cylindrica.* This is probably the first occasion on which these organs have been seen in a species of Rhabditis and at the same time examined with sufficient care to prove them homologous with the amphids in other free-living genera, though they have been previously seen by a few investigators, notably by Dr. deMan in *Rhabditis janeti.* As in *janeti,* so in *cylindrica,* the amphids are relatively more conspicuous in young specimens. In the adult *cylindrica* they are so nearly on the front face of the lip as easily to be overlooked. It seems likely they are present on other Rhabdites but have escaped notice (Fig. 3).

Detached observation extending over many years on nemic genera belonging to various families show the presence in the cardiac region of a very limited number of multipolar ganglion cells of larger size, whose connections indicate the existence of a sympathetic nervous system.

A number of paired organs in nemas are now known to be so widely distributed in the phylum as to make it certain they are distinctive features of the anatomy. I formerly proposed for two of these, one cephalic, the other

cervical, respectively the distinctive names, "amphids" and "deirids," or "derids." Certain lateral organs on the tail are found to be homologous in a wide range of genera; for these I propose the term "phasmids" (Fig. 4). Two of the organs are sensory, at least in part. So far as present knowledge goes, the proposed words may be defined as follows:

Amphids.—Cephalic organs, practically lateral in position; doubtless, in part at least, sensory; often complex, varying very much in size and form in the free-living species, small and obscure in the parasitic species; basically spiral in form externally, the right being a left-hand spiral and the left a right-hand spiral; sometimes slightly different in size on the two sexes. Usually referred to under the indefinite phrase "lateral organs." In parasitic species generally confused with the cephalic papillae.

Deirids.—Lateral organs, presumably sensory, located in the vicinity of the central nervous system; simple, varying from the form of papillae to that of seta-like appendages. Not to be confused with other organs in the same situation, especially with lateral glands, or with lateral papillae that are merely reduced setae. Also spelled "derids."

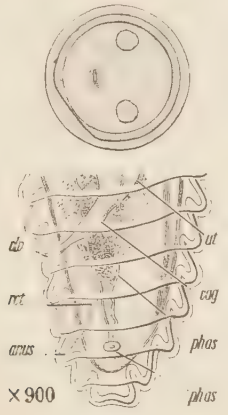

Fig. 4.—Phasmids of Iota. Upper illustration shows a cross section at about midway on the lower illustration. *vlv*, vulva; *rct*, rectum; *anus*, anus; *ut*, uterus; *vag*, vagina; *phas*, phasmid.

Phasmids.—Small, caudal, lateral organs; externally pore-like, often very difficult to observe, whose internal connections are still problematical, but which are not as yet considered sensory—more probably glandular. Often different in the two sexes. Not to be confused with other organs in the same situation, especially with glands belonging to regular lateral series, or with papillae which are merely reduced setae, or with special sensory papillae of the male.

Derivation.—αμφις; opposite, balanced, symmetrical. τερη, τεριη; neck. φάσμα; ghost, phantom—referring to the difficulty with which the organs are observed.

The sixty-fourth meeting was held December 12, 1922.

Dr. Ward discussed his work in Yellowstone Park, dealing with the relation of the pelicans to the native salmon and trout. When they arrive in the spring the pelicans have no parasites but acquire tapeworms by the middle of July. So far as concerns direct transmission the birds get their parasites from the fish, not the fish from the birds. Except for the tapeworms mentioned, parasities in the pelican are rare, only one nematode and one trematode

having been found in the pelicans of the park. The fish in the park are often heavily parasitized by tapeworm larvae, 4 to 5 inches long and quite conspicuous. These are thought by some to be very poisonous, reports of deaths following the eating of infested fish being received but not substantiated. In addition to the internal parasites, the fish had a few copepods on the fins.

Dr. Stiles mentioned the story that in Austria tapeworms from snipe are prepared for food and that according to Rudolphi in Italy a dish of Ligula is prepared, though this is denied by Monticelli and Parona.

Dr. Ransom reported a new case of Gongylonema from man, on the basis of a specimen sent to the Zoological Division of the U. S. Bureau of Animal Industry by Prof. E. Behre of Louisiana State University, Baton Rouge, La. As those heretofore reported, this specimen was found in the mucous membrane of the mouth. He discussed the status of the species recorded by Ward from man and subsequently named *G. hominis* by Stiles. All known specimens of Gongylonema from man have been immature females, which renders difficult their comparison with forms occurring in other animals.

Mr. Godfrey noted the occurrence of the stem nematode in the wild strawberry and fall dandelion in Oregon and Washington and stated that it may be an importation from Europe in ballast soil. Dr. Stiles stated that the Public Health Quarantine Station at Pensacola is built on a ballast dump, which might thus be a fertile field for the study of imported soil nematodes.

Dr. Cobb emphasized the desirability of more careful examination of the labial papillae of parasitic nemas, and noted that amphids, which are universally present in free-living nemas, are being discovered in parasitic forms and that they are functional. Goldschmidt's work on *Ascaris megalocephala* and *A. lumbricoides* indicates that the parasitic worms have structures identical in character with those of free-living forms. The fact that two of the numerous sensory papillae differ markedly from the rest may have taxonomic value. Dr. Ward added that Hetherington reached the conclusion in his work that one pair of the head papillae in parasitic nematodes are different from the others. Dr. Ransom called attention to the amphid-like structures in *Probstmayria vivipara*.

Dr. Bartsch called attention to the term *stenomorph* suggested by him to apply to organisms (shipworms) which because simply of restricted habitat are dwarfed though they reach maturity. Dr. Ransom stated that *Syngamus trachealis* in the chicken might possibly be considered a stenomorph, since in that host it becomes but half as large as when in the turkey, but that other factors are probably involved besides that of the size of the trachea in the two hosts.

Dr. Stiles presented the following note by Dr. N. E. Wayson:

Report of a Case of Dibothriocephalus latus

Nativity Finland, age 30. Departed from Finland at the age of 21 years. Since then has resided in the following states (U. S.): Minnesota, 21-23 year; Montana, 23-26; Idaho, 26-28; Nevada, 28-29; California, 29-30.

Patient remembers evacuating segments of a worm at the age of 16 years. One week before the present examination, he states he evacuated six pieces of tapeworm 16 feet to 20 feet in length. At the present examination numerous Dibothriocephalus ova were found in each microscopic field examined, the specimens having been prepared by centrifugalization. No identification was made of the segments reported by the patient as having been evacuated

Dr. Ward believes there is a species of broad tapeworm native to America, as a tapeworm apparently exactly similar to the European species occurs commonly in both the brown and grizzly bears in Northwest America, in localities where it could hardly be looked upon as a recent introduction from Europe. No exact study of it has yet been made. He has found what he believes to be the larval stage of this tapeworm in salmon at the spawning beds.

The sixty-fifth meeting of the Society was held January 20, 1923.

Dr. Shillinger gave a short note on the migration of *Belascaris marginata* larvae and their presence through prenatal infection in newly born pups. The work which was carried out with the assistance of Dr. Ransom and Miss Cram confirmed observations made by Fülleborn relative to the occurrence of prenatal infections with Belascaris. Eggs from adult Belascaris were cultured in 2 per cent. formalin solution for eight days, and a first dose of the eggs, followed by a second two days later, was given to a pregnant female mongrel fox-terrier. Twelve pups were whelped a week later, of which eight were dead. Six of the eight dead pups had Belascaris larvae in their liver, but none were found in the lungs. The remaining four pups died the next day; of these one appeared to contain no larvae, one had larvae in the lungs, one had larvae in the liver, and in the last, larvae were found in both lungs and liver. No larvae were found in the intestine. Dr. Payne reported two cases of children less than two weeks old passing hookworm eggs regularly. Also a case of a pup about a week old passing ascarid eggs. Dr. Ransom noted that Neveu-Lemaire has reported the finding of *Dictyocaulus filaria* in the lungs

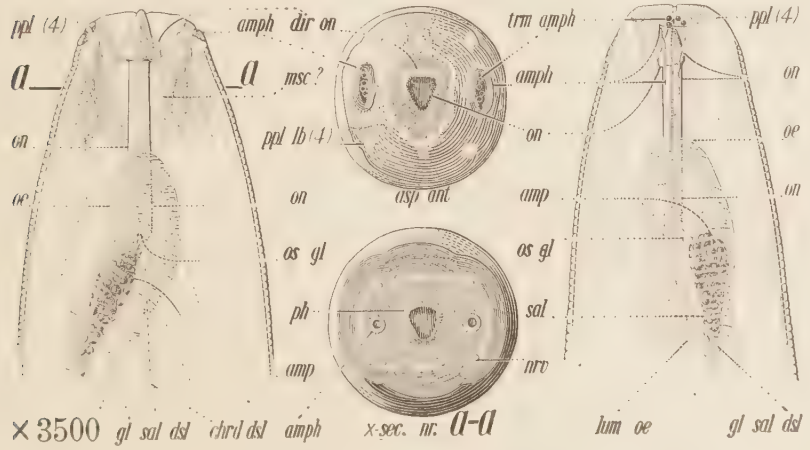

Fig. 5.—Right and left, dorsal and lateral views of head of *Necator americanus* larvae full grown; between them front view of head and optical section near line *aa; ppl,* one of the four labial submedian papillae; *on,* onchium or pharynx; *oe,* esophagus; *gl sal dsl,* dorsal esophageal gland; *chrd dsl,* dorsal chord; *amph,* amphid; *amp,* ampulla of the dorsal esophageal gland; *ph,* pharynx; *on gl sal,* dorsal esophageal gland emptying into lumen of esophagus at the base of the pharynx; *msc?,* contractile fibers?; *dir on,* guiding ring encircling anterior portion of pharynx; *trm amph,* terminals in amphid, presumably end elements of nerve fibers; *sal,* dorsal esophageal gland; *nrv,* nerve passing to submedian labial papilla; *lum oe,* lumen of esophagus. Dorsal and lateral sketches from living specimens; front view and sections from specimens fixed in Flemming's solution and mounted in glycerine jelly.

of both new-born and fetal lambs, and that prenatal infection with Echinococcus has been observed in man by Heyfelder. He also noted that *Belascaris marginata* apparently can pass the capillaries of the lungs more readily than *Ascaris lumbricoides.* Within certain limits, the size of nematode larvae does not appear to be highly important with reference to their ability to cross capillary barriers.

Mr. Augustine discussed the relation of differences in soil to the unsheathing of hookworm larvae. Larvae were placed in various kinds of soils such as clay loam, sand passed through screens of various sizes, and an artificial soil of ground glass. Larvae were recovered for examination after 15 hours. Ground glass gave the largest percentage (over 40 per cent.) of unsheathed larvae, this being a result of cutting of the cuticula by the fragmentary glass. In the case of sand, the percentages of larvae recovered unsheathed varied inversely as the size of the particles until reaching, in the series of gradations, sand passing a screen of 80 meshes to the inch. Finer sand than this gave a smaller percentage of unsheathed larvae. Sheathed and unsheathed larvae appear equally capable of entering the skin and continuing their development in their host.

Dr. Bartsch, with reference to one of the intermediate hosts of Schistosoma, stated that snails of the genus Bullinus do not occur in Africa, the correct name of the genus to which the African snail in question belongs being Isadora. This snail therefore is properly *Isadora contorta* and not *Bullinus contortus*.

Dr. Steiner showed drawings of some Mermithidae, mostly *Mermis albicans*, collected at Falls Church, Va. Many of the females exhibit a pronounced intersexuality, in that they were furnished not only with functional female organs but also in various degrees of development, with a cloacal opening, spicules and genital papillae. These cases of intersexuality, in harmony with Goldschmidt's investigations on the origin of intersexes, indicate crosses among two or more races of the species of Mermis involved, and are of particular interest, also, because of the fact of their occurrence under natural conditions.

Mr. Stoll presented the results of investigations on the relation in cases of hookworm infestation of the egg count to the number of worms present in the patients. Ten patients were selected to include cases of both heavy and light infestations in the test. The results of the investigations showed that on an average, a mature female hookworm passes 9,000 eggs per day, also on the basis of the number of eggs found per gram of feces by the method of egg count employed, that the number of egg-producing female worms present in the intestine can be determined very accurately, about 44 eggs per gram of feces in formed stools representing a single female hookworm in the intestine.

Dr. Cobb presented the results of observations on the larvae of *Necator americanus* together with drawings (Fig. 5) indicating that the full grown larva is provided with a pharyngeal spear, or onchium, which is protrusible through the mouth opening; that the esophagus is provided with three esophageal glands whose nuclei are located in the posterior esophageal swelling; that these glands have three separate ducts two of which empty into the lumen of the esophagus near its middle, and the third at the base of the onchium; and that the lateral cephalic papillae unquestionably belong in the category of organs known as amphids in free-living nematodes. In discussion Dr. Steiner stated that he believes the amphids to be chemical sense organs.

Edward A. Chapin, *Secretary.*

BOOK REVIEWS

Under the title of "De Parasitologische Diagnostiek van de Menschelijke Faeces," Dr. S. L. Brug, director of the Central Laboratory of Military Hygiene, has published at Batavia (Java) an attractive booklet of 75 pages illustrated by 12 plates. A brief but admirable section on methods is followed by a careful description of individual parasites, viz., *Entamoeba tenuis, Endolimax nana, Endolimax williamsi,* Flagellata, *Trichomonas intestinalis, Chilomastix mesnili, Lamblia intestinalis,* Ciliata, *Balantidium coli,* Coccidia and *Blastocystis hominis;* pseudo-parasites, yeast cells and practical hints on handling worm eggs are treated also. As a whole the work is eminently successful in presenting within a limited compass so complete and accurate a survey of the chief human parasites of that region. The plates are clear and life-like, the description brief but good and the work one that will be found useful.

The Report of the Proceedings of the West Indian Medical Conference held in Georgetown, Br. Guiana, from June 28 to July 13, 1921, contains material of sufficient value to parasitologists to justify this belated notice. Filariasis in Barbados and British Guiana is handled at length. Protozoal dysentery, dermal leishmaniasis, malaria, bilharziasis, and hookworm are also discussed in various aspects. A resumé of scientific work published by medical men in Br. Guiana from 1769 to the present time contains important data on parasitology. Also worthy of especial notice is Khalil's paper on the septic tank in the tropics, from a helminthological standpoint. The study demonstrated the fact that helminth eggs pass off into the effluent in large numbers and in living condition, so that these discharges are a potential source of danger and need most careful control.

The London School of Tropical Medicine has published the third series of Collected Papers from the Department of Helminthology covering 1922-1923. Among the ten separate papers, all worthy of note, special mention might be made of Dr. Leiper's review of Medical Helminthology, Part 2, and of Dr. Ortlepp's monograph on the genus Physaloptera.

NOTES

The JOURNAL proposes to begin in the first number of Volume 10 (September, 1923) the publication of a series of illustrated biographical sketches which have long been projected and for which considerable material has already been obtained. Primary attention in these sketches will be given to achievements in the fields of parasitology and medical zoology.

The first article will be a biographical sketch of the distinguished American naturalist Dr. Joseph Leidy and will be illustrated by two portraits and by a facsimile letter. It is peculiarly appropriate to begin the series in this manner since the centenary of Leidy's birth is to be celebrated in September in a fitting manner by the Philadelphia Academy of Natural Sciences and a considerable number of other national scientific organizations with delegates from many national and foreign societies.

News has just come of the death on May 4, 1923, after a long illness of Dr. Arthur Looss, one of the most distinguished pupils of the famous helminthologist Leuckart and intimately associated with him for some years in the work of the department of zoology at Leipzig. Looss went later to Egypt as professor in the medical school at Cairo. At the time of his death he was professor of zoology in the University of Giessen. Looss' work on the morphology and life history of parasitic worms stands out conspicuously by virtue of its finish and accuracy of detail.

NEW HUMAN PARASITES

Plasmodium ovale Stephens 1922.—A malarial parasite in a patient from East Africa examined at the Liverpool School of Tropical Medicine shows certain morphological differences from other species, though resembling somewhat a form described by Ahmed Emin in 1914 under the name of *Plasmodium vivax minuta.* Its periodicity appears to be tertian. The medium-sized forms of the parasite are characteristic; they are non-amoeboid, pigmented, compact, round or oval, resembling the quartan parasite, in red cells showing Schüffner's dots, these cells being normal in size or only slightly enlarged. No gametes were observed. (Annals of Trop. Med., 16:383-388, pl. 16).

Unnamed Intestinal Flagellate.—Chatterjee has found (presumably in India) in the stool of a child suffering from dysentery, numerous actively motile oval flagellates which when stained with iron hematoxylin presented characters unlike those in flagellates previously described. They vary from 4 to 6μ long by 2 to 3μ broad to 14 to 16μ long by 5 to 6μ broad. There is no axostyle nor posterior flagellum; anteriorly are seen a cytostome, flagella and a band-shaped structure representing the nucleus. In the largest specimens 8 free flagella are apparent arranged in 2 clusters of four each. In some specimens only five flagella can be made out, in others only four, originating from basal granules situated within a band-shaped circular ring (representative of the nucleus). The dark stained granules are connected by fibres. (Ind. J. Med. Res., 10: 523-525, pl. 24, 1922).

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The actual dates of issue of Volume IX of THE JOURNAL were as follows:

No. 1, September 21, 1922
No. 2, December 30, 1922
No. 3, May 12, 1923
No. 4, August 22, 1923.